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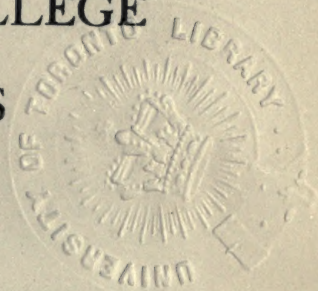








BRYN MAWR COLLEGE  
MONOGRAPHS



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REPRINT SERIES, Vol. VII.

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BRYN MAWR, PENNA., U. S. A.  
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# STUDIES IN SPERMATOGENESIS

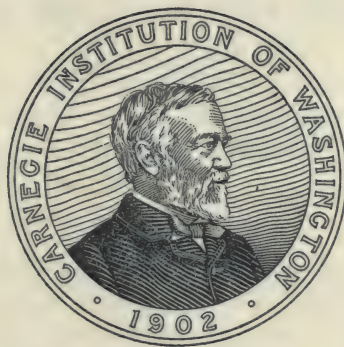
## PART II.

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A COMPARATIVE STUDY OF THE HETEROCHROMO-  
SOMES IN CERTAIN SPECIES OF COLEOPTERA,  
HEMIPTERA AND LEPIDOPTERA, WITH  
ESPECIAL REFERENCE TO

## SEX DETERMINATION.

BY N. M. STEVENS



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## STUDIES IN SPERMATOGENESIS.—II.

A COMPARATIVE STUDY OF THE HETEROCHROMOSOMES IN CERTAIN SPECIES  
OF COLEOPTERA, HEMIPTERA, AND LEPIDOPTERA, WITH ESPECIAL  
REFERENCE TO SEX DETERMINATION.

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By N. M. STEVENS.

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### INTRODUCTION.

In Part I of this series of papers, the spermatogenesis of five species belonging to four different orders of insects was considered. In two species of Orthoptera an "accessory chromosome" was found; in *Tenebrio molitor*, one of the Coleoptera, an unequal pair of chromosomes was described; in the other species no heterochromosomes were discovered. The apparent bearing of the chromosome conditions in *Tenebrio molitor* on the problem of sex determination has led to a further investigation of the germ cells of the Coleoptera. One of the Hemiptera homoptera and two of the Lepidoptera have also been examined for comparison with the Coleoptera and the Hemiptera heteroptera.

### METHODS.

As a result of previous experience with similar material, only two general methods of fixing and staining have been employed: (1) Fixation in Flemming's strong solution or Hermann's platino-aceto-osmic, followed by either Heidenhain's iron-haematoxylin or Hermann's safranin-gentian staining method (Arch. f. mikr. Anat. 1889). (2) Fixation after Gilson's mercurio-nitric formula, followed by iron-haematoxylin, Delafield's haematoxylin and orange G, Auerbach's combination of methyl green and acid fuchsin, or thionin.

The iron-haematoxylin with either mode of fixation gives by far the most satisfactory preparations for general study. The other stains were used mainly for the purpose of distinguishing between heterochromosomes and plasmosomes in resting stages of the nucleus.

## COLEOPTERA.

*Trirhabda virgata* (Family Chrysomelidae).

Two species of *Trirhabda* were found in larval, pupal, and adult stage on *Solidago sempervirens*, one at Harpswell, Maine, the other at Woods Hole, Massachusetts. The adult insects of the two species differ slightly in size and color, the germ cells mainly in the number of chromosomes, *Trirhabda virgata* having 28 and *Trirhabda canadense* 30 in spermatogonia and somatic cells.

In *Trirhabda virgata*, the metaphase of a spermatogonial mitosis (plate VIII, fig. 3) contains 28 chromosomes, one of which, as in *Tenebrio molitor* is very much smaller than any of the others. The maternal homologue of the small chromosome is, as later stages show, one of the largest chromosomes. In *Tenebrio* the unequal pair could not be distinguished in the growth stages of the spermatocytes. In *Trirhabda* it has not been detected in the synizesis stage (fig. 4), but in the later growth stages (figs. 5-7) this pair is conspicuous in preparations stained by the various methods cited above, while the spireme is pale and inconspicuous. The size of the heterochromosome pair varies considerably at different times in the growth period, and in some nuclei (fig. 7) both chromosomes appear to be attached to a plasmosome. The ordinary chromosomes assume the form of rings and crosses in the prophase of the first maturation mitosis (fig. 8), but usually appear in the spindle as dumb-bells or occasionally as tetrads (fig. 10), or crosses (fig. 11). The unsymmetrical pair is plainly seen in figures 9 and 11, but is not distinguishable in a polar view of the metaphase (fig. 13). In the anaphase (figs. 14-16) the larger and the smaller components of the pair separate as in *Tenebrio*. This is, therefore, clearly a reducing division as far as this pair is concerned, and probably for all of the other pairs, though neither the synapsis stage nor the prophase forms are so clear on this point as in some of the other species studied. Figures 17 and 18 show metaphases of the two classes of second spermatocytes, the chromosomes varying somewhat in form in different preparations and even in different cysts of the same preparation. An early anaphase of this mitosis is shown in figure 19; here the small chromosome is already divided. It was impossible to find good polar views of the daughter plates in the two classes of second spermatocytes, but it is evident from figure 19 and other similar views of the second spermatocyte spindle that, as in *Tenebrio*, one-half of the spermatids will contain one of the derivatives of the small chromosome, the other half one of the products of its larger homologue.

Sections of male pupæ were examined for equatorial plates of somatic mitoses. Figure 1 is a specimen of such plates. As might be expected, this figure resembles quite closely the spermatogonial equatorial plate (fig. 3) in number, form, and size of chromosomes, the small one being present in both. Figure 2 is from the follicle of a young egg; here we find 28 chromosomes, but no small one. The chromosome corresponding to the larger member of the unequal pair in the male evidently has a homologue of equal size in the female. The chromosome relations in the male and female somatic cells are therefore the same as in *Tenebrio molitor*, and must have been brought about by the development of a male from an egg fertilized by a spermatozoön containing the small chromosome, and a female from an egg fertilized by a spermatozoön containing the larger heterochromosome.

*Trirhabda canadense.*

In *Trirhabda canadense* the spermatogonial chromosomes are invariably smaller than in *T. virgata*, but similar size relations prevail. The spermatogonial plate (fig. 21) contains 30 chromosomes, 29 large and 1 extremely small. In the growth stages the association of the two unequally paired chromosomes with a rather large plasmosome is more evident than in *T. virgata* (figs. 22-23). In this species the unequal pair is more often found at a different level from the other chromosomes in the early metaphase of the first maturation mitosis (fig. 24), but it later comes into the plate with the other chromosomes (figs. 25-27), and divides earlier than most of the other bivalents (fig. 27). In a polar view of this metaphase the largest chromosome often appears double (fig. 28); in a front view it is a tetrad as in *T. virgata*, figure 10. Figure 29 is the equatorial plate of a metaphase in which the larger component of the unequal pair has been removed in sectioning. The daughter plates of a first spermatocyte in anaphase (fig. 30) show the separation of the components of the heterochromosome pair; and equatorial plates of the resulting two classes of second spermatocytes (fig. 31) show the same conditions. Figures 32 and 33 are prophases of the second division, figure 33 showing the small chromosome ready for metakinesis. It was impossible here also to get good drawings of daughter plates of the second spermatocytes to show the content of the two classes of spermatozoa, but there is no doubt that all of the chromosomes divide in the second mitosis, giving one class of spermatids containing the small chromosome, the other class its larger homologue.

No male somatic cells were found in mitosis, but they would, if found, show the same conditions as in the spermatogonia. One of

many good equatorial plates from egg follicles (fig. 20) shows 30 large chromosomes, indicating an equal pair in place of the unequal pair of the male.

*Chelymorpha argus* (Family Chrysomelidae).

This species was found in larval and adult stages on *Convolvulus arvensis* at Harpswell, Maine, in July and August. It shows the same conditions as *Trirhabda* and *Tenebrio*, so far as the unequal pair of chromosomes is concerned, and is especially favorable for study of synapsis stages. The number of chromosomes in the spermatogonia (plate ix, fig. 36) is 22. Here the components of the unequal pair are the small spherical chromosome and one of the several chromosomes third in size, forming a comparatively small unsymmetrical bivalent (figs. 47-49). The spermatogonia occupy the outer end of each follicle; and next to them comes a layer of cysts in which the chromosomes from the last spermatogonial division are closely massed in the form of short deeply staining loops at one side of the nuclear space (fig. 37). Following this synizesis stage comes one in which some of the short loops have straightened, their free ends extending out into the nuclear space (figs. 38 and 39). Figure 40 shows the nucleus of a slightly later stage in which the free ends of two straightened chromosomes are on the point of uniting. In figures 41 and 42 the point of union of homologous chromosomes is indicated in some cases by a knob, in others by a sharply acute angle. In a slightly later stage (fig. 43), when all of the short loops have straightened and united in pairs, the point of union is no longer visible, all of the loops being rounded at the bend and of equal thickness throughout. My attention was first called to this method of synapsis by the conspicuous difference in number and length of loops in the synizesis stage compared with the later bouquet stage just before the spireme is formed. Following the synapsis stage shown in figure 43 comes one in which the loops lose their polarized arrangement and unite to form a continuous spireme (figs. 44 and 45). In this form, the heterochromosome pair could not be distinguished until the spireme stage, and it is, therefore, uncertain whether these chromosomes remain condensed after the last spermatogonial divisions and are hidden among the massed and deeply staining loops of the synizesis and synapsis stages, or whether they pass through the same synaptic phases as the other chromosomes, condensing and remaining isolated at the beginning of the spireme stage. An early prophase of the first maturation mitosis (fig. 46) shows segments of the spireme longitudinally split, and in some cases transformed into crosses which show a transverse division also. Most of the equal bivalents have the dumb-bell

form in the spindle (figs. 47-49). One is ring-shaped, the ring being formed by union of the free ends of the segment so that the spindle fibers are attached to the middle of each univalent chromosome (fig. 49). This method of ring formation, like that described by Montgomery ('03) for the Amphibia, is of very frequent occurrence in the spermatocytes of the Coleoptera. The dumb-bells are so bent at the ends (fig. 52) that the spindle fibers, here also, are attached at or near the center of each univalent component of a bivalent chromosome, and the separated, univalent chromosomes go to the poles of the spindle in the form of Vs. As in *Tenebrio* the heterochromosome pair is late about coming into the equatorial plate (figs. 47-48), but it does finally take its position with the others (fig. 49) and separates into its component parts somewhat earlier than the other bivalents (figs. 52, 53). Figures 50 and 51 show polar views of the metaphase, the smaller element (*x*) being the unequal pair. The chromosomes in late anaphase are too much crowded to give clear drawings. As in all the beetles so far studied there is no rest stage between the two maturation divisions, but the late anaphase of the first mitosis passes over quickly into the second spindle. Figures 54 and 55 are typical equatorial plates of the second division, one showing the small chromosome (*s*), the other its mate more nearly spherical than the others (*l*). An anaphase including the small chromosome is shown in figure 56. As in the species previously described the spermatozoa are evidently dimorphic.

Female somatic equatorial plates from egg follicles are shown in figures 34 and 35; 22 chromosomes are present and no one is without an equal mate.

*Odontota dorsalis* (Family Chrysomelidae).

*Odontota dorsalis* is a small leaf-beetle found on *Robinia pseudacacia*. The chromosomes are comparatively few in number, 16 in the spermatogonia (figs. 58 and 59), and of immense size when one considers the smallness of the beetle. In some of the spermatogonial cysts many of the chromosomes are V-shaped as in figure 58, while in others all, with the exception of the small one, are rod-shaped as in figure 59, which looks like a hemipteran equatorial plate. The spermatogonial resting nucleus (fig. 60) contains a large plasmosome (*p*), but no condensed chromatin. The synizesis and synapsis stages are similar to those in *Chelymorpha* (figs. 61 and 62). The spireme stage (figs. 63, 64) contains, in addition to the pale spireme, a very conspicuous group consisting of a large plasmosome with a large and a small chromosome attached to it. In the prophase, before the nuclear membrane has disappeared, this group is easily distinguished from the other

dumb-bell and ring-shaped bivalents (figs. 65-67). In preparations much destained (fig. 67) the small chromosome component of the group retains the stain longer than the larger one. The spindle in prophase (fig. 68) is much elongated and the 8 chromosomes are often spread out upon it so as to be easily counted. In the early metaphase the parachute-like heterochromosome group is always nearer one pole of the spindle (plate x, figs. 69 and 70). The equatorial plate often shows both the larger component of the pair and the plasmosome (fig. 71). Figures 72-74 show the metakinesis of the heterochromosome bivalent. In figure 74 the two unequal elements are completely separated and the plasmosome has disappeared. The equatorial plates of the two resulting kinds of second spermatocytes appear in figures 75 and 76. In the anaphase of the second division all of the chromosomes are divided quantitatively as may be seen in figures 77 and 78. A few dividing male somatic cells were found in the walls of the testis. Figure 57 (plate ix) is an equatorial plate from one of these. The chromosomes are like those of the spermatogonia (figs. 58 and 59), 15 large and 1 small. No dividing female somatic cells were found.

A few drawings of developing spermatids are given to show the transformations of a peculiar body which seems to be characteristic of insect spermatids. Figure 79 is a very young spermatid showing only diffuse chromatin in the nucleus. The nucleus soon enlarges (fig. 80) and a large dense body (*n*) appears which stains like chromatin with various staining media. A little later (fig. 81) the chromatin forms a homogeneous, more or less hemispherical or sometimes crescent-shaped mass which stains an even gray in iron-haematoxylin. In addition the nucleus contains a body (*n*) smaller than in the preceding stage, but staining the same. As the nucleus condenses and elongates to form the sperm head, a light region containing this deeply staining body is seen on one side (figs. 82, 83). A little later the body is divided into two, which appear sometimes spherical (fig. 84), sometimes elongated (fig. 85). As the sperm head elongates still more, approaching maturity, these bodies diminish in size (figs. 86, 87) and ultimately disappear. A cross section of the sperm head at such a stage as figure 87 shows the chromatin in crescent shape with material which stains very little within (fig. 88). The chromatin-like body described above was observed in *Tenebrio* in a stage corresponding to figure 81, and it was thought that the larger body seen in some cases and the smaller one in others might be the larger and smaller heterochromosomes, but a study of this element in more favorable material disproves that supposition by showing that the different sizes are merely different phases in the evolution of the body. Throughout its

history it stains like dense chromatin, and my only suggestion as to its origin is that it seems, from a study of this and other species of beetles, to be a derivative of the chromatin of the spermatid, increasing in size for a time, then decreasing, and finally breaking up into granules and dissolving in the karyolymph. Whether it has any function connected with the development of the spermatozoon, or whether it is merely material rejected from the chromosomes, as in many cases in oögenesis, one can only surmise.

In one testis a peculiar abnormality was found. In all of the perfect spermatogonial plates two small chromosomes were present (figs. 89, 90). Nineteen such plates were counted in five different cysts. All of the equatorial plates of the first spermatocytes showed 8 chromosomes, as usual. In a few favorable growth stages (fig. 91) the two small chromosomes were seen to be combined with the larger heterochromosome and a plasmosome, and one first spermatocyte spindle was found in which the same combination could be clearly seen (fig. 92). All of the second spermatocyte metaphases in which a small chromosome occurred, contained two small ones, making 9 in all (fig. 93). The others contained 8 large chromosomes, as usual. The only explanation suggested by the conditions is that somewhere in its history, the small chromosome had undergone an extra division, and that ever afterward the two products behaved like the one small heterochromosome of a normal individual. The chief interest in this abnormality centers in the fact that the two small chromosomes of this specimen behave exactly like the usual single one, emphasizing the individuality of this particular heterochromosome. Both evidently have the same individual characteristics and affinities as the one in other cases.

*Epilachna borealis* (Family Coccinellidae).

*Epilachna borealis* was found in abundance on squash vines at Woods Hole, Massachusetts, in September. The testes, unlike those of most of the Coleoptera, consist of many free follicles similar to those of the Orthoptera. The germ glands were rather far advanced, but some good spermatogonial and spermatocyte cysts were found. In figure 94, a spermatogonial metaphase, the small chromosome is shown with 17 larger ones. The heterochromosome pair appears in condensed form in the spireme stage (fig. 95), and again in the first maturation spindle (figs. 96, 97). The varying forms of the ordinary chromosomes are shown in figure 98. Figures 99 and 100 are equatorial plates of the first mitosis. The unequal pair is shown by itself in figure 101, and the separation of the heterochromo-

somes is seen in figure 102. Equatorial plates of the second division, one containing the small chromosome (*b*), are shown in figure 103. A prophase of the same division (fig. 104) proves that the small chromosome divides quantitatively like the others. It was interesting to find here and there in this material whole cysts in which the nuclei were like those described by Paulmier ('99) for *Anasa tristis* (plate XIII, fig. 14) as cells which were being transformed to serve as food for the growing spermatids (figs. 105, 106). The only occasional appearance of these cysts seems to me to preclude their being a special dispensation to furnish the spermatids with nutrition during their transformation. Their appearance and size make me suspect that they are giant spermatids due to the failure of one of the spermatogonial or spermatocyte mitoses. The smaller chromatin body seems to correspond to that described for the spermatids of *Odontota dorsalis*.

*Euphoria inda* (Family Scarabaeidae).

Of *Euphoria inda* only one male was captured, but the numerous testes furnished abundant material in desirable stages. The spermatogonial equatorial plate (fig. 107) contains 20 chromosomes of which the two smallest (*l* and *s*) form the unequal pair. The resting spermatogonium contains a two-lobed plasmosome (fig. 108). The growth stages are similar to those in *Tenebrio* in showing no distinct bouquet stage, but there is a spireme stage in which the heterochromosome pair is clearly seen (fig. 109). Figure 110 (plate XI) is an early prophase, and figure 111 one in which the unequal pair appears with a tetrad and several dumb-bell forms. The prophase of the spindle, as in *Odontota*, is much elongated (fig. 112). In figures 113-116 the small heterochromosome pair is shown in various positions with reference to the other chromosomes of the metaphase of the first spermatocyte. Figure 117 shows it more deeply stained than the others in the equatorial plate. This pair divides in advance of the others, and the larger and smaller elements are plainly seen nearer the poles in anaphase than the other univalent chromosomes (figs. 118-120). Daughter plates of the first spermatocyte are shown in figure 121, and equatorial plates of the second spermatocyte in figure 123. Figure 122 shows the telophase of the first division with the spindle for the second division forming. In figures 124 and 125 we have daughter plates of the two classes of second spermatocytes, showing the content of the two equal classes of dimorphic spermatozoa, as this was shown in *Tenebrio*. Figures 126 and 127 are anaphases showing the division of the heterochromosomes (*l* and *s*). Figures 128-130 are early stages in the development of the spermatid showing the chromatin nucleolus (*n*) in various phases.

*Blepharida rhois* (Family Chrysomelidae).

The testes were rather too far advanced when this material was collected and no dividing spermatogonia were present. The growth stages (figs. 131, 132) show a faintly staining spireme and a heterochromosome group similar to that of *Odontota*, a large and a small chromosome attached to a large plasmosome. The spireme appears to go directly over by condensation and segmentation into the dumb-bell-shaped figures seen in the first maturation spindle (figs. 133, 134), though cross-shaped bivalents occasionally occur (fig. 135). The heterochromosome pair, slightly separated by plasmosome material, is usually found at the periphery of the plate (figs. 133-136). Figure 137 is an exceptional anaphase in which the heterochromosome elements are not mingled with the polar masses of chromatin. Figures 138 *a* and *b* are equatorial plates of the second mitosis, and figures 139 and 140 are pairs of daughter plates from second spermatocytes showing again the dimorphism of the spermatozoa as to their chromatin content. As in several of the forms studied, material was collected for examination of the somatic cells, but no favorable cases of mitosis were to be found.

*Silpha americana* (Family Silphidae).

Only one male of this species was secured, but the large testes gave all stages in abundance. The chromosomes, however, were very small and too numerous, 40 in the spermatogonia (fig. 141). The small chromosome is, nevertheless clearly distinguished in many of these plates (*s*). The resting spermatogonium contains one very large plasmosome and often one or two smaller ones (fig. 142, *p*). The unequal pair is seen in the growth stages (figs. 143, 144), and may frequently be seen outside of the equatorial plate of the first spermatocyte spindle (fig. 146). In favorable sections it may also be found in the plate among the other bivalents (fig. 147). Figure 145 is a prophase showing the bivalent chromosomes still connected by linin fibers. An equatorial plate of the first division is shown in figure 148, and a pair of corresponding plates of the second spermatocyte in figure 149. The small heterochromosome divides in the second spindle in advance of the others as seen in figure 150. Therefore, although this form is not especially favorable for detailed study on account of the large number of small chromosomes, the conditions are evidently the same as in the other species described—an unsymmetrical heterochromosome bivalent in the first spermatocyte, giving rise by the second maturation division to equal numbers of dimorphic spermatozoa, one class receiving the large heterochromosome, the other class the small one.

*Doryphora decemlineata* (Family Chrysomelidae).

*Doryphora decemlineata* has been the most difficult one of the collection to work out satisfactorily. The chromosomes in the spermatogonial plates were in most cases much tangled, and the behavior of the heterochromosome pair was such as to suggest an "accessory chromosome" rather than an unequal pair. Abundant material for the study of somatic cells was at hand, but nothing favorable could be found in the sections.

Two spermatogonial plates, containing 36 chromosomes, are shown in figures 151 and 152 (plate XII). The small heterochromosome (*s*) is slightly elongated. The synizesis and synapsis stages are especially clear. The chromosomes, after the last spermatogonial mitosis go over immediately into a synizesis stage consisting of a polarized group of short loops, which later straighten and unite in pairs (figs. 153 and 154). From these loops are formed the spireme (figs. 155-158), which splits and segments, producing various cross, dumb-bell, and ring forms (figs. 159-163). As in most of the other species of Coleoptera, the unequal pair is not distinguishable until the spireme stage. Figure 162 is an unusual prophase in which all of the equal pairs show a longitudinal split as well as a transverse constriction, and the larger heterochromosome (*l*) is also split. Figure 163 shows a somewhat later and more common prophase in which the unequal pair, one ring, crosses, and dumb-bells may be seen. This figure, as well as figures 164-168, show the unequal pair in various relations to the other chromosomes. This pair in *Doryphora* consists of a large V-shaped chromosome with a small spherical one attached to it in different positions. When the small one is behind the V, the group has the appearance of an orthopteran "accessory."

Figures 169-171 show the separation of the two elements outside of the equatorial plate, while in figure 168 the unequal pair is in line with the other chromosomes. In figure 172, an anaphase, the unequal elements are barely separated, while the metakinesis of the other pairs is much further advanced.

Figures 173 and 174 are equatorial plates of the first division, one showing only the larger element of the heterochromosome pair (fig. 174, *x*), the other both elements (fig. 173, *l* and *s*). In the late anaphase (fig. 175) the larger heterochromosome is often seen outside of the polar mass, reminding one again of the "accessory" in the Orthoptera. Occasionally it is found in some other isolated position (fig. 176). Equatorial plates of the second division show the same conditions as in the other species; some contain the larger heterochromosome, others the smaller one (fig. 177, *a* and *b*). It was impos-

sible to draw anaphases of the second division from a polar view and the lateral view showed nothing unusual, merely the longitudinal division of all of the chromosomes.

The spermatids show some interesting variations from the other species which have been examined. In figures 178 and 179 we have telophases of the second spermatocyte, showing centrosome and archoplasm (fig. 178) and certain masses of deeply staining material in the cytoplasm (fig. 179,  $a_1$ ). Figures 180 and 181 are young spermatids showing the archoplasm from the second spindle ( $a_2$ ) and a smaller, more deeply staining mass ( $a_1$ ), derived from the irregular masses of the earlier stage (fig. 179,  $a_1$ ). In figures 182 and 183, the axial fiber has appeared and the larger mass of archoplasm ( $a_2$ ) is being transformed into a sheath. The other body remains unchanged. During the following stages this smaller archoplasmic body ( $a_1$ ) lies in close contact with the axial fiber and sheath ( $a_2$ ), and gradually decreases in size (figs. 184-186) until it disappears in a slightly later stage. The acrosome seems to develop directly out of the cytoplasm. The enigmatical body ( $a_1$ ), which is probably archoplasm from the first maturation spindle, as it is not found in the cytoplasm of the first spermatocyte, may serve as nutriment for the developing axial fiber. The sperm head has a peculiar triangular form, staining more deeply on two sides.

#### Miscellaneous Coleoptera.

Considerable material from the spruce borers was collected at Harpswell, Maine, but the species were not identified. Although these insects were in the pupa stage, most of the testes were too old. There were no dividing spermatogonia and few spermatocyte mitoses. Most of the spermatocytes contained 10 chromosomes, one of which was plainly an unequal pair. In a few testes the number was 11, indicating that pupæ of two species had been collected. Figure 187 shows the metaphase of first spermatocyte mitosis with the unequal pair in metakinesis. Figures 188 and 189 are first spermatocyte equatorial plates of the two species, containing 10 and 11 chromosomes respectively. Figure 190 is a first spermatocyte spindle in anaphase, showing the unequal pair behind the other chromosomes. Figure 191 is an equatorial plate from a second spermatocyte, showing the small chromosome. In figure 192 are shown several of the bivalent chromosomes, including the unsymmetrical pair, from nuclear prophases of the first division, all from the same cyst.

*Adalia bipunctata* (family Coccinellidæ), the common lady beetle, has a very conspicuous pair of unequal heterochromosomes, as may be

seen in figures 193-197 (plate XIII). This would seem to be a favorable form for determining the chromosome conditions in somatic cells, but no clear equatorial plates were found in either larvæ or pupæ.

In *Cicindela prineriana* (family Cicindelidæ) there are 18 chromosomes in the spermatogonium (fig. 198), one being small. The heterochromosome group is blended into a vacuolated sphere in growth stages (figs. 199, 200). In the metaphase of the first division it is trilobed, or tripartite (fig. 201), and in metakinesis, a small spherical chromosome separates from a much larger V-shaped one (fig. 202). Equatorial plates of first and second spermatocytes are shown in figures 203 and 204. Whole cysts of giant first spermatocytes were found both in growth stages (fig. 205) and prophases (fig. 206). Here the heterochromosome group is plainly double (fig. 205), and the conditions observed must have been due to the failure of a spermatogonial mitosis to complete itself.

Several of the Carabidæ have been studied, and the material, though not especially favorable, is interesting in that some members of the family have an unequal pair of heterochromosomes, others an odd one. *Chlænium æstivus* (figs. 207-212), *Chlænium pennsylvanicus* (figs. 213-215), and *Galerita bicolor* (fig. 216) have the unequal pair, while *Anomoglossus emarginatus* (figs. 217-223) has an odd heterochromosome ( $x$ ), which behaves exactly like the larger heterochromosome in other carabs.

In the Elateridæ and Lampyridæ we also have examples of the second type with the odd chromosome. Two Elaters, species not determined (figs. 224-229 and 230-235), have each 19 chromosomes in the spermatogonia (figs. 224 and 230), and in the first spermatocyte division an odd chromosome ( $x$ ) which is in each case the smallest. In the first of these Elaters, the female somatic number was determined to be 20 (fig. 229). In the second Elater the pairs of second spermatocytes, containing 9 and 10 chromosomes respectively in the two cells, were in nearly every case connected as shown in figure 235, one pair of chromosomes not having separated completely in the first mitosis. Of *Ellychnia corrusca* (family Lampyridæ) only the spermatogonial equatorial plate, containing 19 chromosomes ( $x$ , the odd one) is given, as no material in maturation has yet been obtained, and a comparative study of the germ cells of the Elateridæ and Lampyridæ will be made as soon as suitable material can be secured.

In addition to the species of Coleoptera described here, two others, *Coptocyclus aurichalcea* and *Coptocyclus guttata* have been studied by one of my students and the results published elsewhere (Nowlin, '06). In both an even number of chromosomes (22, 18) was found in the sper-

matogonia, one being very small and forming with a larger one an unequal pair which remained condensed during the growth stage and separated into its larger and smaller components in the first spermatocyte mitosis. The result of maturation, as in the other species here described and in *Tenebrio molitor*, is dimorphism of the spermatozoa. The method of synopsis in *Coptocycla* is like that described for *Chelymorpha argus*.

#### HEMIPTERA HOMOPTERA.

##### *Aphrophora quadrangularis*.

The abundance of *Aphrophora* at Harpswell, Maine, in June and July, 1905, suggested that it might be well to examine at least one more of the Hemiptera homoptera for comparison with the many species of Hemiptera heteroptera which have been recently reexamined by Wilson ('05, '05, '06).

The larvæ only were collected, as they gave all the desired stages for a study of the spermatogenesis, and also oögonia and synyzesis and synopsis stages of the oöcytes. In the first collections the testes were dissected out, but the many free follicles break apart so easily that the later material was prepared by cutting out the abdominal segments which contained the reproductive organs, and fixing those without dissection. The same methods of fixation and staining were employed as for the Coleoptera. Hermann's safranin-gentian method was especially effective with this material.

In *Aphrophora* the follicles of each testis are free, forming a dense cluster, each follicle being connected with the vas deferens by a short duct. The very young follicles are spherical, the older ones ovoid in form. The primary spermatogonia (plate XIV, fig. 237)—very clear cells with a lobed nucleus which stains slightly—occupy the tip of the follicle. Next to these comes a layer of cysts of secondary spermatogonia which are conspicuous for their deeper staining quality (fig. 238). There appears to be no plasmosome in either class of spermatogonia. Figure 239 is the equatorial plate of a secondary spermatogonium. There are 23 chromosomes, two of which are conspicuously larger than the others and evidently form a pair. The odd one is one of the three next in size.

Next to the secondary spermatogonia are cysts of young spermatocytes, whose nuclei show a continuous spireme and an elongated deeply staining chromatin rod which is the odd chromosome (fig. 240). This is often more elongated than in the figure and more or less wormlike in appearance. A pair of smaller chromatin masses may sometimes be detected at this stage, and are readily found a little

later (fig. 241) when the nucleus has enlarged and the spireme has become looser and stains less deeply. Here the odd chromosome is more condensed, or shortened, and split. There is no synizesis and no polarized or bouquet stage, but the nuclei of all of the spermatocytes contain a continuous spireme throughout the growth stage. Synapsis must occur at the close of the last spermatogonial mitosis before the spireme is formed. Figures 242 and 243 show a slightly later growth stage. The form and connection of the "*m*-chromosome" pair (Wilson, '05<sub>b</sub>) comes out clearly here. Figure 244, from a safranin-gentian preparation, shows both the odd chromosome and the *m*-chromosomes. Some time before the first mitosis, the spireme splits and the pairs of granules embedded in linin are wonderfully distinct, both in iron-haematoxylin and safranin-gentian preparations (fig. 245). The *m*-chromosomes have here formed a precocious tetrad (*m*). Figure 246 is a similar stage from a safranin-gentian preparation. Figures 247 and 248 show the condensation of chromatin granules to form tetrads of various sizes, still embedded in the linin spireme. As these tetrads come into the spindle without losing their elongated form, it is evident that each one consists of two longitudinally split chromosomes united end to end in synapsis and separated in the first maturation mitosis, which is therefore reductional. The odd chromosome and the *m*-chromosomes show no longitudinal split in these figures, but they may appear as in figure 249. Occasionally one of the tetrads takes the form of a cross (fig. 249). In this figure the split "accessory" (*x*) lies against the nuclear membrane and the archoplasmic material for the spindle is seen along one side of the nucleus. It is certain here that the spindle fibers come from extranuclear material, not from nuclear substance, as Paulmier ('99) describes for *Anasa tristis*.

Figures 250 and 251 show the first maturation mitosis as it usually appears in sections from mercurio-nitric material stained with iron-haematoxylin. The odd chromosome is always more or less eccentric and is attached by a spindle fiber to one pole. In Hermann material, considerably destained, the tetrads and the odd chromosome appear as in figures 252, 253, and 254, the tetrads being in position for a transverse division. The odd chromosome is always so placed that its longitudinal split is at right angles to the axis of the spindle, as though it were to divide in this mitosis. It does not do so, however, but goes to one daughter cell, always lagging behind, as is shown in figures 255 and 256. Figures 257, *a* and *b*, are polar plates of the first mitosis with 11 and 12 chromosomes, respectively, and figures 258, *a*, *b*, and *c*, show the polar plates (*a* and *c*) each containing

11 chromosomes, and the odd chromosome at a different level (*b*). The latter is a view of the anaphase which one often gets at three foci in one section. Figures 259, *a* and *b*, are equatorial plates of the second mitosis with 11 and 12 chromosomes respectively. Figure 260 shows a side view of the second spindle in metaphase, and figure 261 in anaphase. Figures 262 and 263 are daughter plates from two spindles showing the chromosome content of the two equal classes of spermatozoa, one class containing 11 ordinary chromosomes, the other 11 ordinary chromosomes plus the odd heterochromosome, for the odd chromosome divides with the others in the second spindle as in Orthoptera (McClung and Sutton).

In figures 264 and 265 (plate xv) are seen the telophase of the two kinds of second spermatocytes, one (fig. 265) showing the divided odd chromosome, which continues to stain more deeply after the others have become diffuse. All of the spermatids (figs. 266-268) contain, in the early stages of development, a body (*n*) which stains like chromatin, but increases in size from a small granule in the telophase (figs. 264, 265) to the large dense body (*n*) seen in figure 267. This is probably homologous with the chromatin nucleolus described for the spermatids of the Coleoptera. In addition to this, in one-half of the spermatid nuclei there is a condensed mass of chromatin which is evidently the derivative of the odd chromosome of the spermatogonia and spermatocytes (figs. 267 and 268, *x*). In common with the spermatids of other Hemiptera these show two masses of archoplasm, the larger of which forms the sheath (*s*) of the axial fiber of the tail, and the smaller the acrosome (*a*). The axial fiber grows out directly from the centrosome, on either side of which there is a dense band forming the lateral boundary of the middle piece. It will be seen that the odd chromosome of *Aphrophora* is in its behavior precisely like the typical Orthopteran "accessory" of McClung, and similar to the odd chromosome of the Coleoptera.

In various parts of the young male larvæ dividing cells were found and the number 23 determined (fig. 269). Turning now to the female larvæ to determine the somatic number, the oögonia proved to be more favorable for counting. Twenty-four chromosomes were present in equatorial plates of oögonial mitoses (fig. 270), thus confirming Wilson's results for the *Anasa* group of the Hemiptera heteroptera.

In examining sections of female larvæ stained with safranin-gentian-violet, I was surprised to see a very marked polarized or bouquet stage and to find among the loops something resembling the odd chromosome of the growing spermatocytes. It was difficult to get a clear view of this body as it lay within the loops. In one section of a

slightly earlier stage before synapsis, there were found two pairs of chromosomes (fig. 271,  $x_1$ ,  $x_2$ , and  $m_1$ ,  $m_2$ ) which were stained with safranin in contrast with the violet spireme. These two pairs I interpret as being (1) the homologues of the pair of  $m$ -chromosomes, which remain condensed during the growth stage of the spermatocytes, and (2) a pair of heterochromosomes corresponding to the odd chromosome of the male. Various combinations of these heterochromosomes are shown in figures 272-277. Figures 278 and 279 were taken from mercurio-nitric material stained with iron-hæmatoxylin. In section 278 the "bouquet" was cut through, showing the bivalent corresponding to the larger pair in figure 271, and in figure 279 this element is seen behind the paler loops. The history of these two pairs of heterochromosomes, which have not, so far as I know, been found before in oöcytes, should be followed up in older ovaries, and related species should be examined for similar phenomena.

#### LEPIDOPTERA.

##### Cacoecia and Euvanessa.

I had no intention of making an extended study of the spermatogenesis of the Lepidoptera, but was interested to see if anything corresponding to the heterochromosomes of other orders could be found. The material studied was the testes of the larvæ of *Cacoecia cerasivorana* and *Euvanessa antiopa*. The number of chromosomes is large, but the equatorial plates are diagrammatically clear. In both species 30 chromosomes are found in both first and second spermatocytes. In both, one chromosome is larger (figs. 290 and 293,  $x$ ). In the growth stage (figs. 283, 284) there is a two-lobed body (or sometimes two separate spherical bodies) which seems to correspond in size to the larger pair of chromosomes in the first spermatocyte. In iron-hæmatoxylin preparations this pair is often obscured by parts of the spireme which are tangled around it. In safranin-gentian preparations it stains, not like a plasmosome, but red like the heterochromosomes, while the spireme is violet. The staining reaction at least suggests that this equal pair of chromosomes, which may be traced through the synzesis stage (fig. 280), synapsis stage (figs. 281, 282), growth stages (figs. 283, 284), and prophases (figs. 285-287), into the first spermatocyte spindle (figs. 288, 290), and on to the second spermatocyte (figs. 289, 291, 292), is an equal pair of heterochromosomes comparable to the equal pair of "idiochromosomes" found by Wilson in *Nezara* ('05). As the various stages are practically the same in *Euvanessa antiopa*, but somewhat clearer in *Cacoecia*, only one figure is given for *Euvanessa*—the equatorial plate of the first spermatocyte (fig. 293).

## SUMMARY OF RESULTS.

(1) An unequal pair of heterochromosomes has been found by the author in 19 species of Coleoptera belonging to 8 families :

FAMILY.	SPECIES.
I. Buprestidæ ... ..	Two spruce-borers, species not determined.
II. Carabidæ .....	{ 1. <i>Chlœnius æstivus</i> . 2. <i>Chlœnius pennsylvanicus</i> . 3. <i>Galerita bicolor</i> .
III. Chrysomelidæ .....	{ 1. <i>Blepharida rhois</i> . 2. <i>Chelymorpha argus</i> . 3. <i>Coptocycla aurichalcea</i> . 4. <i>Coptocycla guttata</i> . 5. <i>Doryphora decemlineata</i> . 6. <i>Odontota dorsalis</i> . 7. <i>Trirhabda virgata</i> . 8. <i>Trirhabda canadense</i> .
IV. Cicindelidæ .....	<i>Cicindela primeriana</i> .
V. Coccinellidæ .....	{ <i>Adalia bipunctata</i> . <i>Epilachna borealis</i> .
VI. Scarabæidæ .....	<i>Euphoria inda</i> .
VII. Silphidæ .....	<i>Silpha americana</i> .
VIII. Tenebrionidæ .....	<i>Tenebrio molitor</i> .

(2) An odd chromosome, which behaves during the growth stage of the first spermatocytes like the " accessory " of the Orthoptera, has been found in 4 species of Coleoptera,\* belonging to 3 families:

FAMILY.	SPECIES.
I. Carabidæ .....	<i>Anomoglossus emarginatus</i> .
II. Elateridæ .....	Two Elaters ; species not determined.
III. Lampyridæ .....	<i>Ellychnia corrusca</i> .

(3) In most of the species of Coleoptera examined, the unequal pair or the odd chromosome remains condensed during the growth period of the first spermatocyte, like the " accessory " of the Orthoptera and the various heterochromosomes of the Hemiptera.

(4) Several of these species of Coleoptera have a synizesis stage in which the spermatogonial number of short loops is massed at one side of the nucleus. This is followed by a synapsis stage in which the loops straighten and unite in pairs, forming longer loops which soon

\*Aug. 20, 1906.—Since this paper was prepared, 19 other species of Coleoptera have been studied. Of these, 17 have an unequal pair of heterochromosomes in the spermatocytes. Six belong to the Chrysomelidæ, making 14 of that family that have been examined. Representatives of 4 new families—Melandryidæ, Lamiinæ, Meloidæ, Cerambycinæ—have been studied. In only two species,—1 Elater and 1 Lampyrid—has the odd chromosome been found in place of the unequal pair. No species of Coleoptera has yet been examined in which one or the other of these two types of heterochromosomes does not occur in the spermatocytes. Of the 42 species of Coleoptera whose germ cells have been studied, 85.7 per cent are characterized by the presence of an unequal pair of heterochromosomes in the male germ cells, 14.3 per cent by the presence of an odd chromosome.

spread out in the nuclear space, and, with the exception of the heterochromosomes, unite to form a continuous spireme.

(5) In several of the species of Coleoptera and in Aphrophora, it has been shown that a body staining like chromatin develops in the spermatids, increasing in size for a time, then breaking up into granules and disappearing. This body evidently has no relation to the heterochromosomes, as it is the same for all of the spermatids. Its staining qualities suggest that it may be material derived from the chromosomes. It is finally dissolved in the karyolymph.

(6) In iron-haematoxylin preparations the heterochromosomes of the Coleoptera vary greatly in their staining properties during mitosis. In some species they stain exactly like the ordinary chromosomes, in others the larger one of the unequal pair holds the stain more tenaciously than the others and also than its smaller mate, and this is true in several cases where the heterochromosome is smaller than the other chromosomes, which destain more readily. The odd chromosome of the Elaters stains less deeply than the others in the first spermatocyte. In the growth stage they stain more deeply, as a rule, than the spireme, with iron-haematoxylin or thionin, stain red with safranin-gentian and green with Auerbach's methyl green-fuchsin combination.

(7) *Aphrophora quadrangularis* agrees with the *Anasa* group of Hemiptera heteroptera in having a pair of *m*-chromosomes and an odd chromosome in the spermatocytes, but differs from many of that group in that the odd chromosome divides in the second mitosis instead of the first. It also differs from other known forms in exhibiting heterochromosomes in certain stages of the oöcytes.

(8) The two species of Lepidoptera examined have an equal pair of heterochromosomes.

COMPARISON OF RESULTS IN DIFFERENT SPECIES OF  
COLEOPTERA.

In number of chromosomes there is great variation, the smallest number (16) having been found in *Odontota dorsalis*, and the largest (40) in *Silpha americana*. The difference in size is also very marked, as may be seen by comparing the spermatogonial plates in figures 3 and 58 with those shown in figures 94 and 141.

No other species of the Tenebrionidæ has yet been secured, and all of the other beetles examined differ in a marked degree from *Tenebrio molitor* in the growth stages of the spermatocytes. While in *Tenebrio* the chromatin stains very dark throughout the growth stage, and the unequal pair can not be distinguished until the prophase of division ('05, plate VI, figs. 171-180), in most of the others there are very distinct synizesis and synapsis stages, following the last spermatogonial mitosis, then a spireme stage in which the condensed unequal pair of heterochromosomes or the odd chromosome is conspicuous in contrast with the pale spireme, whether the preparation is stained with iron-hæmatoxylin, gentian, or thionin. In *Tenebrio molitor*, the unequal pair behaved in every respect like the other bivalent chromosomes. In the other forms, though it behaves during the two maturation divisions like the symmetrical bivalents, it remains condensed during the growth period like the "accessory" of the Orthoptera, the odd chromosome, "*m*-chromosomes," and "idiochromosomes" of the Hemiptera. In several cases the heterochromosomes of the Coleoptera are associated with a plasmosome (figs. 22, 23, 63, 132, 158, 217), as is often true in other orders. This peculiar pair of unequal heterochromosomes varies considerably in size during the growth stage in some of the species studied, but changes very little in form, differing in this respect from the "accessory" in some of the Orthoptera (McClung, '02) and from the large idiochromosome in some of the Hemiptera (Wilson, '05).

The odd chromosome, so far as it has been studied, behaves precisely like the larger member of the unequal pair without its smaller mate (figs. 219, 220, 226, 233). In the growth stage it remains condensed and either spherical or sometimes flattened against the nuclear membrane (figs. 217, 225, 231). In the first maturation mitosis it is attached to one pole of the spindle, does not divide, but goes to one of the two second spermatocytes (figs. 233, 235). In the second spermatocyte it divides with the other chromosomes, giving two equal classes of spermatids differing by the presence or absence of this odd chromosome.

All of the evidence at hand leads to the conclusion that in the Coleoptera, the univalent elements of all the pairs, equal and unequal, separate in the first spermatocyte mitosis and divide quantitatively in the second. In this respect the behavior of the chromosomes in this order appears to be much more uniform than in the Orthoptera and Hemiptera.

#### COMPARISON OF THE COLEOPTERA WITH THE HEMIPTERA AND LEPIDOPTERA.

As has been seen above, the conditions in the Coleoptera, so far as the heterochromosomes are concerned, correspond very closely in final results with those in the Hemiptera heteroptera and the Orthoptera. In minor details these chromosomes are less peculiar in the Coleoptera than in either of the other orders. Even condensation during the growth stage is not universal, and synapsis of the heterochromosomes apparently occurs simultaneously with that of the ordinary chromosomes, instead of being delayed, as in many of the Hemiptera heteroptera.

*Aphrophora* (Hemiptera homoptera) agrees with the *Anasa* group of the Hemiptera heteroptera in having a pair of condensed *m*-chromosomes, in the growth stage, but this pair is already united in synapsis when first seen. It differs from *Anasa*, but agrees with *Banasa* and *Archimerus* in exhibiting a typical odd chromosome which goes to one pole without division in the first spermatocyte, and divides with the other chromosomes in the second spermatocyte. The odd chromosome in this species of Hemiptera, therefore, behaves like that in the Coleoptera and Orthoptera. The most interesting points in the results of this study of the germ cells of *Aphrophora* is the discovery of two pairs of condensed chromosomes in certain phases of the growth stages of the oöctyes. This has not been shown to be the case in any other species of Hemiptera, so far as I can ascertain. It is now evident that in the Heteroptera homoptera there are at least two distinct classes as to behavior of chromosomes. In one class we have the Aphids (Stevens, '05 and '06) and Phylloxera (Morgan, '06) in which no heterochromosomes have been found, while in the other class are such forms as *Aphrophora* with both a pair of *m*-chromosomes and a typical odd heterochromosome.

The two species of Lepidoptera examined indicate that here we may have conditions comparable to those in *Nezara*—an equal pair of heterochromosomes whose only apparent peculiarity is their condensed form during the growth stage. Doubtless the results of other investigators will soon throw more light on the heterochromosomes of this order.

## GENERAL DISCUSSION.

It will be seen from the foregoing that the results obtained in the study of the germ cells of *Tenebrio molitor* have been confirmed in full for several species of Coleoptera, and in part for 19\* different species belonging to 8\* families. It has also been shown that a different type of Coleopteran spermatogenesis exists in at least 3 families, where an odd chromosome like that in the Orthoptera occurs in place of the unequal pair. In all of these insects the spermatozoa are distinctly dimorphic, forming two equal classes, one of which either contains one smaller chromosome or lacks one chromosome.

The most difficult part of the work has been the determination of the somatic number of chromosomes in the male and female. In some cases suitable material has been lacking; in others, though material was abundant, no metaphases could be found in which the chromosomes were sufficiently separated to be counted with certainty. In three species (in addition to *Tenebrio molitor*) where the unequal pair is present, the female somatic cells have been shown to contain the same number of chromosomes as the spermatogonia, but an equal pair in place of the unequal pair of the male. In two new cases the male somatic number and size have been shown to be the same as in the spermatogonia. In one of the Elateridæ, where the spermatogonial number is 19, the female somatic number is 20, and in *Aphrophora* the numbers in male and female cells are respectively 23 and 24. No exception has been found to the rule established by previous work on the Coleoptera (Stevens, '05) and on the Hemiptera (Wilson, '05 and '06), that (1) in cases where an unequal pair is present in the male germ cells, it is also present in the male somatic cells, but is replaced in the female by an equal pair, each component being equal in volume to the larger member of the unequal pair, and (2) in cases where an odd chromosome occurs in the male, a pair of equal size are found in the female. It is therefore evident that an egg fertilized by a spermatozoon (1) containing the small member of an unequal pair or (2) lacking one chromosome, must develop into a male, while an egg fertilized by a spermatozoon containing the larger element of an unequal pair of heterochromosomes or the odd chromosome must produce a female.

Whether these heterochromosomes are to be regarded as sex chromosomes in the sense that they both represent sex characters and determine sex, one can not decide without further evidence.

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\*Aug. 20, 1906.—36 species belonging to 12 families. See note, p. 49.

Comparison of the two types in Coleoptera, especially where, as in the Carabidæ, both occur in one family, has suggested to me that here it is possible that the small chromosome represents not a degenerate female sex chromosome, as suggested by Wilson, but some character or characters which are correlated with the sex character in some species and not in others. Assuming this to be the case, a pair of small chromosomes might be subtracted from the unequal pair, leaving an odd chromosome. The two types would then be reduced to one. It may be possible to determine the validity of this suggestion for particular cases by observation or experiment.

Since the first of this series of papers was published, there have appeared three important papers by Prof. E. B. Wilson, bearing on the problem of sex determination in insects. These papers are based on a study of many species of the Hemiptera heteroptera. These insects fall into two classes—one in which a pair of "idiochromosomes," usually of different size, remain separate and divide quantitatively in the first spermatocyte, conjugate and then separate in the second maturation mitosis; and another class in which an odd chromosome—the "heterotropic" chromosome—divides in one of the maturation mitoses, but not in the other. Wilson regards the odd chromosome as the equivalent of the larger of the "idiochromosomes," its smaller mate having disappeared. In the somatic cells of the former class he finds in the male the unequal pair, in the female an equal pair, the smaller chromosome being replaced by an equivalent of the larger "idiochromosome." In the latter class the male somatic cells contain the odd number, the female somatic cells and oögonia an even number, the homologue of the odd chromosome of the male being present and giving to the female one more chromosome than are found in the male.

In his latest paper Wilson ('06) makes a variety of suggestions as to sex determination. He shows that if the "idiochromosomes" and the heterotropic chromosome be regarded as sex chromosomes in the double sense that they both bear sex characters and determine sex, the following scheme accounts for the observed facts in all cases where an unequal pair or an odd heterochromosome have been found:

Sperm.	Egg.
I. $\left\{ \begin{array}{l} \text{Large } \sigma^{\text{♂}} \text{ "idiochromosome"} \\ \text{or} \\ \text{Odd chromsome.} \end{array} \right\}$	$+ \text{Large } \text{♀} \text{ sex chromosome} = a \text{ } \text{♀}$
II. $\left\{ \begin{array}{l} \text{Small } \text{♀} \text{ "idiochromosome"} \\ \text{or} \\ \text{No sex chromosome} \end{array} \right\}$	$+ \text{Large } \sigma^{\text{♂}} \text{ sex chromosome} = a \text{ } \sigma^{\text{♂}}$

Here we know that such a combination of gametes must occur to give the observed results, but we are not certain that we have a right to attribute the sex characters to these particular chromosomes or in fact to any chromosomes. It seems, however, a reasonable assumption in accordance with the observed conditions. The scheme also assumes either selective fertilization or, what amounts to the same thing, infertility of gametic unions where like sex chromosomes are present. It also assumes that the large female sex chromosome is dominant in the presence of the male sex chromosome, and that the male sex chromosome is dominant in the presence of the small female sex chromosome. Or, it might rather be said that these are not really assumptions, but inferences as to what must be true if the heterochromosomes are sex chromosomes. This theory of sex determination brings the facts observed in regard to the heterochromosomes under Castle's modification of Mendel's Law of Heredity ('99).

The question of dominance is a difficult one, especially in parthenogenetic eggs and eggs which are distinctly male or female before fertilization. It may be possible that the sex character of the egg after maturation is always dominant in the fertilized egg, as appears to be the case in these insects (see scheme). Conditions external to the chromosomes may determine in certain cases, such as *Dinophilus*, which sex character shall dominate in the growing oöcyte, and maturation occur accordingly. It is evident that this reasoning would lead to the conclusion that sex is or may be determined in the egg before fertilization, and that selective fertilization, or infertility of gametic unions containing like sex characters, has to do, not with actual sex determination, but with suitable distribution of the sex characters to future generations. If both sex characters are present in parthenogenetic eggs, as appears to be the case in aphids and phylloxera, dominance of one or the other must be determined by conditions external to the chromosomes, for we have both sexes at different points in the same line of descent without either reduction or fertilization.

Wilson suggests as alternatives to the chromosome sex determinant theory according to Mendel's Law, (1) that the heterochromosomes may merely transmit sex characters, sex being determined by protoplasmic conditions external to the chromosomes; (2) That the heterochromosomes may be sex-determining factors only by virtue of difference in activity or amount of chromatin, the female sex chromosome in the male being less active. The first of these alternatives is an attempt to cover such cases as *Dinophilus*, *Hydatina*, and *Phylloxera* with large female and small male eggs. Here Morgan's ('06) suggestion as to degenerate males seems much to the point. The male sex

character, having become dominant in certain eggs at an early stage, may, from that time on, determine the kind of development. As to the second alternative, I see no reason for supposing that the small heterochromosome of a pair is in any different condition, as to activity, from the large one. The condensed condition may not mean inactivity, but some special form of activity. And, moreover, it has been shown that in certain stages of the development of the oöcyte of one form, *Aphrophora quadrangularis*, there are pairs of condensed chromosomes corresponding to those of the spermatocyte, so that there would hardly seem to be any basis for Wilson's attempt to associate the difference in development of male and female germ cells with activity or inactivity of chromosomes, as indicated by condensed or diffuse condition of the chromatin.

On the whole, the first theory, which brings the sex determination question under Mendel's Law in a modified form, seems most in accordance with the facts, and makes one hopeful that in the near future it may be possible to formulate a general theory of sex determination.

This work has been done in connection with a study of the problem of sex determination, but, whatever may be the final decision on that question, it brings together a mass of evidence in favor of the belief in both morphological and physiological individuality of the chromosomes, as advocated by Boveri, Sutton, and Montgomery. It also gives the strongest kind of evidence that maternal and paternal homologues unite in synapsis and separate in maturation, leaving the ripe germ cells pure with regard to each pair of characters.

BRYN MAWR COLLEGE, June 7, 1906.

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[The figures were all drawn with Zeiss oil-immersion 2mm., oc. 12, and have been reduced one-third, giving a magnification of 1,000 diameters.]

PLATE VIII.

*Trirhabda virgata* (Family Chrysomelidae).

- FIG. 1. Equatorial plate from somatic tissues of a male pupa, 27 large chromosomes, 1 small one.
2. Equatorial plate from an egg follicle, 28 large chromosomes.
  3. Equatorial plate of spermatogonium, 27 large chromosomes, 1 small one.
  4. First spermatocyte, synizesis stage.
  5. First spermatocyte, early spireme stage, showing unequal pair of chromosomes.
  - 6-7. First spermatocyte, later growth stages.
  8. First spermatocyte, prophase.
  - 9-12. First spermatocyte, metaphase.
  13. First spermatocyte, equatorial plate.
  - 14-15. First spermatocyte, anaphase, showing separation of the elements of the unequal pair (*l* and *s*).
  16. First spermatocyte, daughter plates.
  17. Second spermatocytes, equatorial plates.
  18. Second spermatocytes, equatorial plates showing V-shaped chromosomes.
  19. Second spermatocyte, early anaphase, the small chromosome in metakinesis.

*Trirhabda canadense*.

20. Equatorial plate from egg follicle, 30 large chromosomes.
21. Equatorial plate of spermatogonium, 29 large chromosomes, 1 small one.
22. First spermatocyte, growth stage showing the heterochromosome group.
23. Heterochromosome group. *p* = plasmosome, *l* = large heterochromosome, *s* = small heterochromosome.
- 24-27. First spermatocyte, metaphase.
28. First spermatocyte, equatorial plate.
29. First spermatocyte, equatorial plate, small member of the unequal pair only present.
30. First spermatocyte, daughter plates.
31. Second spermatocytes, equatorial plates.
- 32-33. Second spermatocytes, prophase.







## PLATE IX.

*Chelymorp̄ha argus* (Family *Chrysomelidæ*).

- FIGS. 34-35. Equatorial plates from egg follicles, 11 equal pairs, no small chromosome.  
36. Equatorial plate of spermatogonium, 21 large chromosomes, 1 small one.  
37. First spermatocyte, synizesis stage.  
38-40. First spermatocyte, synapsis stage.  
41-43. First spermatocyte, bouquet stage after synapsis.  
44-45. First spermatocyte, spireme stage showing the unequal pair of heterochromosomes.  
46. First spermatocyte, prophase.  
47-49. First spermatocyte, metaphase.  
50-51. First spermatocyte, equatorial plates,  $\alpha$  the heterochromosome pair.  
52. First spermatocyte, showing metakinesis of the unequal pair.  
53. First spermatocyte, anaphase.  
54-55. Second spermatocyte, equatorial plates.  
56. Second spermatocyte, anaphase.

*Odontota dorsalis* (Family *Chrysomelidæ*).

57. Equatorial plate of male somatic cell from walls of the testis, 15 large chromosomes, 1 small one.  
58-59. Equatorial plates of spermatogonia, 15 large chromosomes, 1 small one.  
60. Resting nucleus of spermatogonium, showing plasmosome ( $\phi$ ).  
61. First spermatocyte, synizesis stage.  
62. First spermatocyte, synapsis stage.  
63-64. First spermatocyte, spireme stage, showing the larger and smaller heterochromosome associated with a plasmosome.  
65-68. First spermatocyte, prophase.







## PLATE X.

*Odontota dorsalis*.

Figs. 69-70. First spermatocyte, metaphase.

71. First spermatocyte, equatorial plate.

72. First spermatocyte, metaphase, showing metakinesis of the heterochromosomes.

73-74. First spermatocyte, anaphase.

75-76. Second spermatocyte, equatorial plates.

77. Second spermatocyte, showing metakinesis of the small chromosome (*s*).

78. Second spermatocyte, prophase, showing chromosomes longitudinally split.

79-80. Young spermatids, *n* the chromatin nucleolus.

81-87. A series of stages in the development of the sperm head, showing the various phases in the history of the chromatin nucleolus (*n*).

88. Cross-sections of nearly mature sperm heads.

89-90. Equatorial plates of spermatogonia of abnormal individual, 15 large chromosomes, 2 small ones.

91. First spermatocyte from same testis, spireme stage, showing 2 small chromosomes associated with 1 large one and a plasmosome.

92. First spermatocyte from the same testis, metaphase showing a similar heterochromosome group.

93. Second spermatocyte from same testis, equatorial plate, showing 2 small chromosomes.

*Epilachna borealis* (Family Coccinellidae).

94. Equatorial plate of spermatogonium, 17 large chromosomes and 1 small one.

95. First spermatocyte, spireme stage, showing the unequal pair.

96-97. First spermatocyte, late prophase.

98. First spermatocyte, metaphase, showing chromosomes of different forms.

99-100. First spermatocyte, equatorial plate.

101. Unequal heterochromosome pair from a metaphase.

102. First spermatocyte, anaphase; ordinary chromosomes stippled to show more clearly the metakinesis of the unequal pair.

103. Second spermatocyte, equatorial plates.

104. Second spermatocyte, prophase.

105-106. Abnormal giant spermatids, probably in process of degeneration.

*Euphoria inda* (Family Scarabaeidae).

107. Equatorial plate of spermatogonium, 20 chromosomes. The 2 smallest are the unequal pair of heterochromosomes (*l* and *s*).

108. Resting spermatogonium, showing plasmosome (*p*).

109. First spermatocyte, spireme stage.







## PLATE XI.

*Euphoria inda.*

- FIGS. 110-111. First spermatocyte, prophases.  
 112-113. First spermatocyte, late prophase.  
 114-116. First spermatocyte, metaphase.  
 117. First spermatocyte, equatorial plate,  $x$  the unequal pair.  
 118-120. First spermatocyte, anaphase.  
 121. First spermatocyte, daughter plates.  
 122. Second spermatocyte, prophase.  
 123. Second spermatocyte, equatorial plates.  
 124-125. Second spermatocyte, daughter plates of the two classes.  
 126-127. Second spermatocyte, anaphase.  
 128-130. Spermatids,  $n$  the chromatin nucleolus.

*Blepharida rhois* (Family Chrysomelidae).

- 131-132. First spermatocyte, spireme stages, showing the heterochromosome group.  
 133-135. First spermatocyte, beginning of metakinesis.  
 136. First spermatocyte, equatorial plate,  $x$  the unequal pair.  
 137. First spermatocyte, late anaphase, showing the heterochromosomes  $l$  and  $s$ .  
 138. Second spermatocyte, equatorial plates.  
 139-140. Second spermatocyte, daughter plates of the two classes.

*Silphu americana* (Family Silphidae).

141. Equatorial plate of spermatogonium, 40 chromosomes—39 large, 1 small.  
 142. Resting nucleus of spermatogonium, showing 2 plasmosomes ( $p$ ).  
 143-144. First spermatocyte, spireme stage.  
 145. First spermatocyte, prophase.  
 146-147. First spermatocyte, metaphase.  
 148. First spermatocyte, equatorial plate.  
 149. Second spermatocyte, equatorial plates.  
 150. Second spermatocyte, showing metakinesis of the small chromosome.







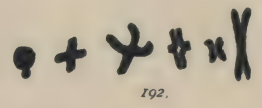
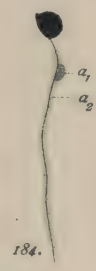
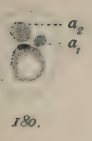
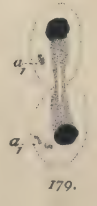
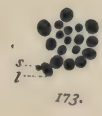
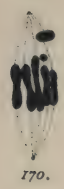
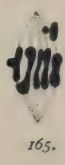
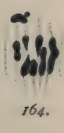
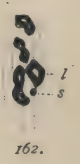
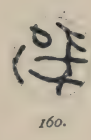
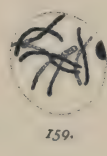
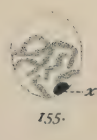
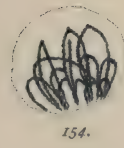
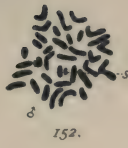
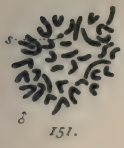
## PLATE XII.

*Doryphora decemlineata* (Family Chrysomelidae).

- FIGS. 151-152. Equatorial plates of spermatogonia, 36 chromosomes—35 large, 1 small.  
153. First spermatocyte, synizesis stage.  
154. First spermatocyte, synapsis stage.  
155-158. First spermatocyte, spireme stages.  
159. First spermatocyte, spireme segmented and split.  
160-163. First spermatocyte, prophases.  
164-171. First spermatocyte, metaphase.  
172. First spermatocyte, anaphase.  
173-174. First spermatocyte, equatorial plates.  
175-176. First spermatocyte, late anaphase.  
177. Second spermatocyte, equatorial plates.  
178-179. Second spermatocyte, telophase,  $a_1$ , archoplasmic material.  
180-186. Spermatids in different stages;  $a_1$ , archoplasmic material from first spermatocyte spindle,  $a_2$  archoplasmic material from second maturation spindle.

*Spruce-borers* (Family Buprestidae).

187. First spermatocyte, metaphase.  
188-189. First spermatocyte, equatorial plates of two species, 10 and 11 chromosomes.  
190. First spermatocyte, anaphase.  
191. Second spermatocyte, equatorial plates containing 9 large chromosomes and 1 small one.  
192. Chromosomes from prophase of the first spermatocyte, all from the same cyst.







## PLATE XIII.

*Adalia bipunctata* (Family Coccinellidae).

- FIG. 193. Equatorial plate of spermatogonium, 20 chromosomes—19 large, 1 small.  
 194. First spermatocyte, spireme stage,  $x$  the heterochromosome group.  
 195. First spermatocyte, metaphase.  
 196. First spermatocyte, equatorial plate.  
 197. Second spermatocyte, equatorial plates.

*Cicindela primeriana* (Family Cicindelidae).

198. Equatorial plate of spermatogonium, 20 chromosomes—19 large, 1 small.  
 199. First spermatocyte, spireme stage,  $x$  the heterochromosome group.  
 200. First spermatocyte, prophase.  
 201. First spermatocyte, metaphase,  $x$  the unequal pair in tripartite form.  
 202. First spermatocyte, showing metakinesis of the heterochromosomes ( $l$  and  $s$ ).  
 203. First spermatocyte, equatorial plate.  
 204. Second spermatocyte, equatorial plates.  
 205. Giant spermatocyte, spireme stage, heterochromosome group double the usual size.  
 206. Giant spermatocyte, prophase.

*Chlenius aestivus* (Family Carabidae).

207. First spermatocyte, spireme stage, showing the unequal pair associated with a large plasmosome.  
 208. First spermatocyte, metaphase.  
 209–210. First spermatocyte, beginning of metakinesis.  
 211. First spermatocyte, equatorial plate, 17 chromosomes.  
 212. First spermatocyte, anaphase, showing elongated centrosome and diverging univalent chromosomes.

*Chlenius pennsylvanicus*.

213. First spermatocyte, spireme stage.  
 214. First spermatocyte, equatorial plate,  $x$  the unequal bivalent.  
 215. First spermatocyte, late prophase.

*Galerita bicolor* (Family Carabidae).

216. Equatorial plate of spermatogonium, 30 chromosomes—29 large, 1 small.

*Anomoglossus emarginatus* (Family Carabidae).

217. First spermatocyte, growth stage,  $x$  the odd chromosome.  
 218. First spermatocyte, prophase.  
 219–220. First spermatocytes, metaphase,  $x$  the odd chromosome.  
 221. First spermatocyte, equatorial plate.  
 222. First spermatocyte, daughter plates containing 18 and 19 chromosomes, respectively.  
 223. Second spermatocytes, equatorial plates.

*Elater I* (Family Elateridae, species not determined).

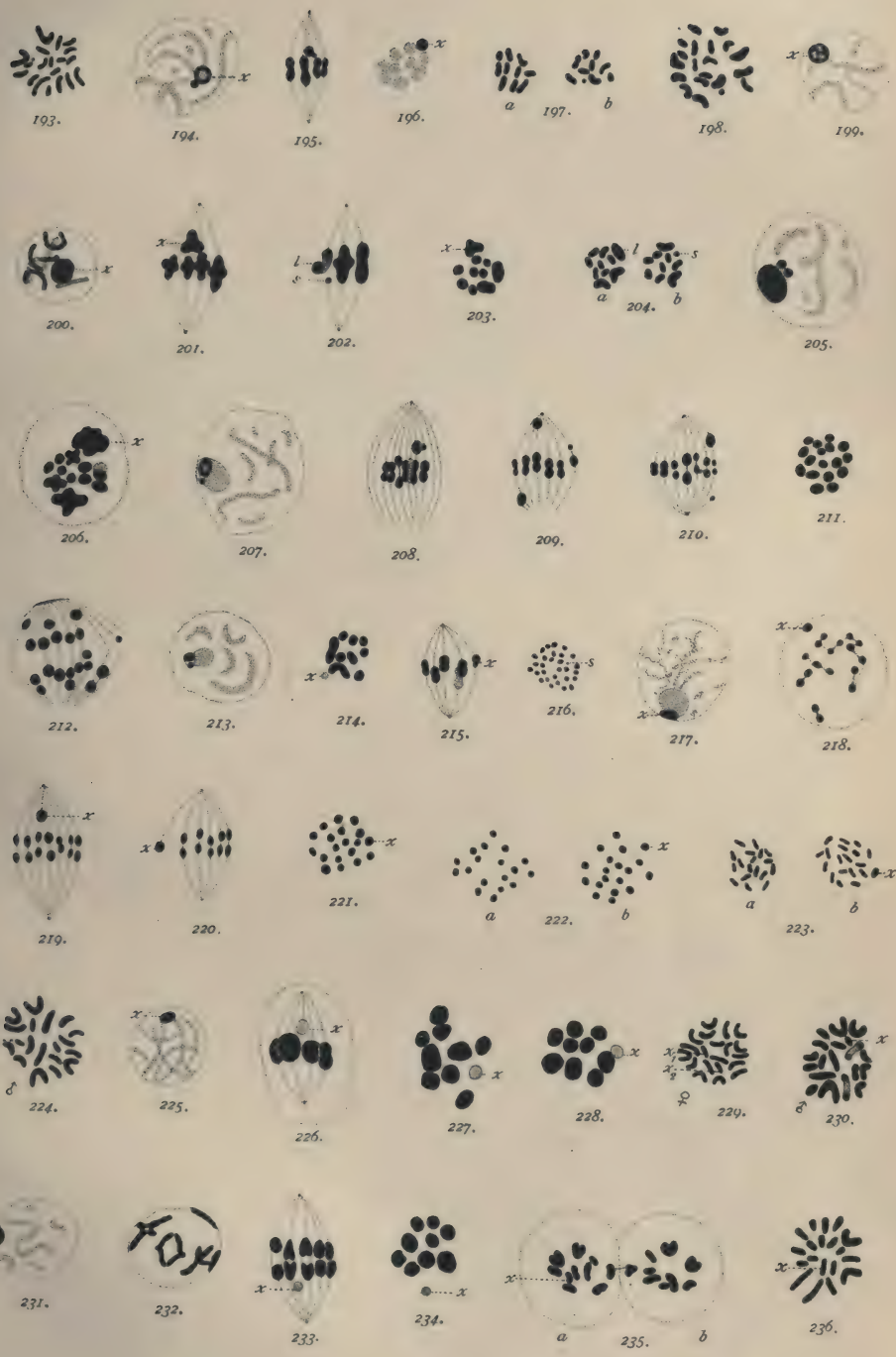
224. Equatorial plate of spermatogonium, 19 chromosomes,  $x$  the odd one.  
 225. First spermatocyte, spireme stage,  $x$  the odd chromosome.  
 226. First spermatocyte, metaphase.  
 227. First spermatocyte, prophase.  
 228. First spermatocyte, equatorial plate.  
 229. Equatorial plate from egg follicle, 20 chromosomes,  $x_1$  and  $x_2$  the pair corresponding to  $x$  in the spermatogonium.

*Elater II* (Species not determined).

230. Equatorial plate of spermatogonium, 19 chromosomes,  $x$  the odd one.  
 231. First spermatocyte, spireme stage.  
 232. First spermatocyte, prophase.  
 233. First spermatocyte, beginning of metakinesis.  
 234. First spermatocyte, equatorial plate,  $x$  the odd chromosome.  
 235. A pair of second spermatocytes in metaphase, two chromosomes connected,  $x$  the odd chromosome.

*Ellychnia corrusca* (Family Lampyridae).

236. Equatorial plate of spermatogonium, 19 chromosomes.



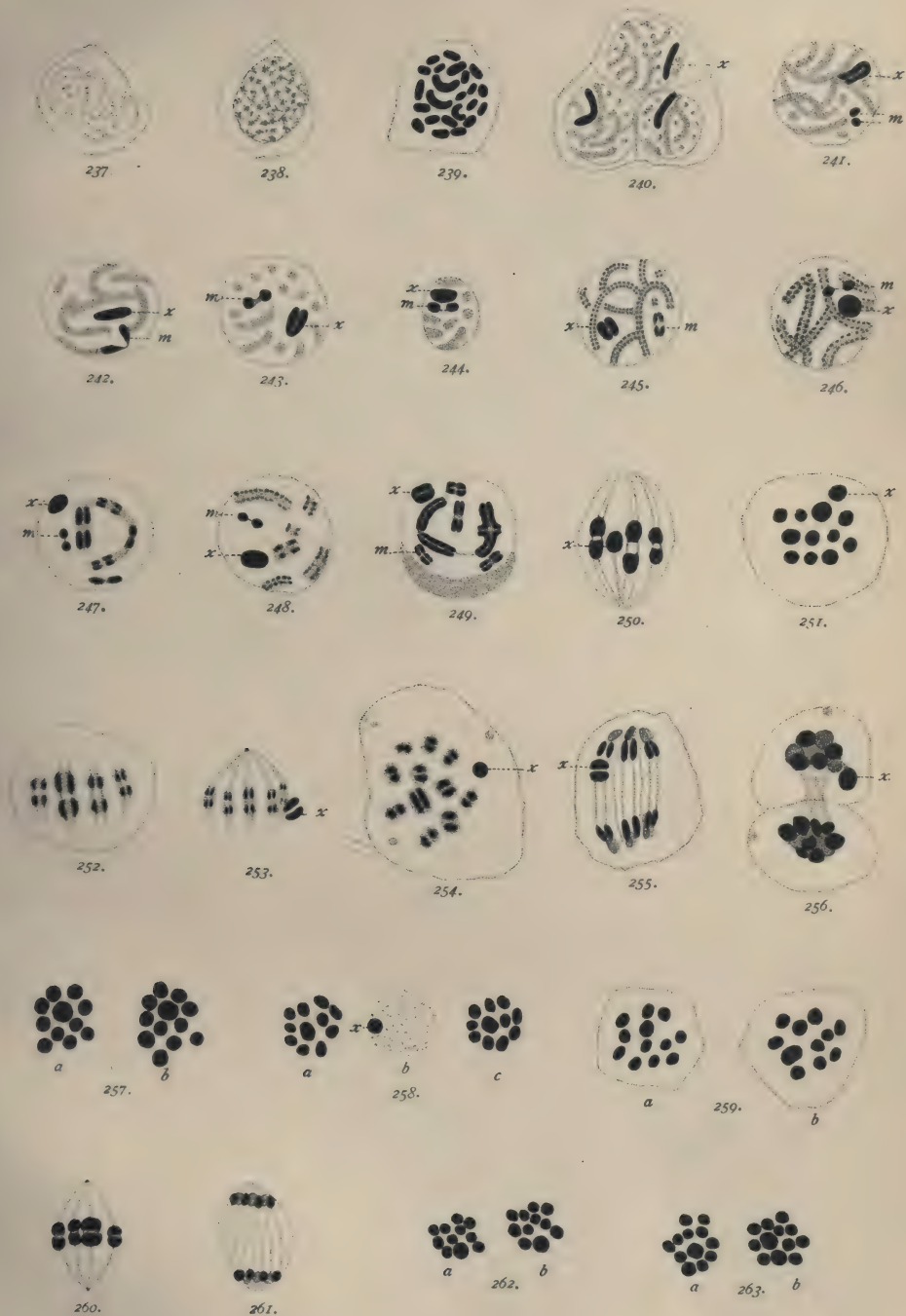




## PLATE XIV.

*Aphrophora quadrangularis* (Hemiptera homoptera).

- FIG. 237. Resting primary spermatogonium with lobed nucleus.  
238. Resting secondary spermatogonium, with nucleus staining much more deeply.  
239. Equatorial plate of secondary spermatogonium, 23 chromosomes.  
240. First spermatocytes, very early growth stage,  $x$  the odd chromosome.  
241-243. First spermatocyte, later spireme stages, showing the odd chromosome ( $x$ ) and a pair of  $m$ -chromosomes ( $m$ ).  
244. Similar stage from a safranin-gentian preparation.  
245. First spermatocyte, split-spireme stage,  $x$  the odd chromosome,  $m$  the  $m$ -chromosome tetrad.  
246. Similar stage from a safranin-gentian preparation.  
247-248. First spermatocyte, condensation of chromatin granules to form tetrads in the linin spireme.  
249. Later tetrad stage.  
250-251. First spermatocytes, metaphase from mercurio-nitric material.  
252-254. Similar stages from Hermann material, showing longitudinal split in both the bivalents, and the odd chromosome ( $x$ ).  
255. First spermatocyte, anaphase.  
256. First spermatocyte, telophase.  
257. First spermatocyte, daughter plates containing 11 and 12 chromosomes, respectively.  
258. First spermatocyte,  $a$  and  $c$  daughter plates, each containing 11 chromosomes,  $x$  the odd chromosome at a different level ( $b$ ).  
259. Second spermatocyte, equatorial plates of the two classes.  
260. Second spermatocyte, metaphase.  
261. Second spermatocyte, anaphase.  
262-263. Second spermatocyte, daughter plates of the two classes.







## PLATE XV.

*Aphrophora quadrangularis.*

- FIGS. 264-265. Second spermatocyte, telophase, showing chromatin nucleolus ( $n$ ) and the products of division of the odd chromosome ( $x$ ).
266. A spermatid containing the chromatin nucleolus ( $n$ ).
- 267-268. Spermatids containing both the chromatin nucleolus ( $n$ ) and the odd chromosome ( $x$ ),  $a$  the acrosome.
269. Equatorial plate from a somatic cell of a male larva, 23 chromosomes.
270. Equatorial plate of an oögonium, 24 chromosomes.
271. Resting nucleus of a young oöcyte before synapsis, showing two pairs of condensed chromosomes, corresponding in size to the  $m$ -chromosomes and the odd chromosome of the spermatocytes.
- 272-277. Sections of nuclei of oöcytes, showing one or more of these heterochromosomes, from safranin-gentian preparations.
- 278-279. Bouquet stage from iron-haematoxylin preparations, showing the heterochromosome bivalent ( $x$ ).

*Cacæcia cerasivorana (Lepidoptera).*

280. First spermatocyte, synizesis stage, showing 2 condensed chromosomes ( $x_1$  and  $x_2$ ).
- 281-282. First spermatocyte, synapsis stage.
- 283-284. First spermatocyte, growth stages.
285. First spermatocyte, prophase.
- 286-287. First spermatocyte, later prophases, showing the heterochromosome pair ( $x$ ).
288. First spermatocyte, metaphase.
289. Second spermatocyte, metaphase.
290. First spermatocyte, equatorial plate.
- 291-292. Second spermatocyte, equatorial plates.

*Euvanessa antiopa (Lepidoptera).*

293. First spermatocyte, equatorial plate.





# A STUDY OF THE SPERMATOGENESIS OF COPTOCYCLA AURICHALCEA AND COPTOCYCLA GUTTATA, WITH ESPECIAL REFERENCE TO THE PROBLEM OF SEX-DETERMINATION

BY

W. N. NOWLIN

WITH TWO PLATES

Very little work on the germ cells of the Coleoptera had been done until last year, when the form *Tenebrio molitor* was investigated by Dr. N. M. Stevens. The results obtained were so suggestive from the standpoint of sex-determination that she is at present engaged in examining a number of species of this order of insects.

The following paper is a contribution to this series of investigations, and I take pleasure in acknowledging my indebtedness to Dr. Stevens, both for material used and for direction in the work.

## I. MATERIAL AND METHODS

The beetles under discussion are *Coptocycla aurichalcea* and *Coptocycla guttata*, both collected from *Convolvulus arvensis* at Woods Hole, Mass., September 5, 1905.

The testes were fixed in Flemming's strong solution, Hermann's platino-aceto-osmic, and Gilson's mercurio-nitric, each of which gave excellent results.

Heidenhain's iron-hæmatoxylin method was used for most of the preparations. Other stains, employed principally for chemical tests, were thionin, Delafield's hæmatoxylin and orange G, and with the material fixed in Hermann's and Flemming's fluids, the safranin-gentian stain of Hermann. The iron-hæmatoxylin slides were often counterstained with orange G.

## II. OBSERVATIONS

The testes of these beetles consist of many follicles radiating from a central duct, the vas deferens, into which are poured the spermatozoa. We should expect then to find ripe spermatozoa at the inner end of the follicles and spermatogonia at the outer, with the intervening stages between. This is true in most cases but it is by no means an inviolable rule. Cysts containing ripe spermatozoa may be found mingled with those containing first or second spermatocytes, and second spermatocytes may occur in the same cyst with the first. The classification then cannot be made by the location but purely from the character of the cell itself.

1. *Coptocycla aurichalcea*

The equatorial plate of a spermatogonium of *Coptocycla aurichalcea* contains twenty-two chromosomes, most of which are V-shape, and radially arranged with the concave side outward (pl. I, Fig. 1). Near the center usually, though it may take any other position, is a very small chromosome, which is the small member of an "idiochromosome" group, and while we cannot here be sure of its mate we are safe in assuming it to be one of the five smaller chromosomes. An attempt was made to arrange the chromosomes in pairs according to form and size, but it soon became evident that they differed in different plates; the form, whether rod-shaped or V-shaped, depending largely on position with respect to the other chromosomes. As is unusual the behavior of the differential chromosomes in the spermatogonium is in every respect like that of the others: each divides longitudinally and passes to the poles, where condensed masses are formed.

The earliest spermatocyte shows a true condensation stage or bouquet stage [synizesis of McClung ('05)], in which the chromatin is in threads closely looped together at one side of the nuclear space (Fig. 2). The number of loops is here very evidently more than the reduced number and probably each loop corresponds to a spermatogonial chromosome. In a slightly later stage the loops straighten until many free ends are seen above the condensed mass (Figs. 3 and 5). Each of these strands appears to be a single

chromosome, and this idea is strengthened when in the next phase the distal ends are seen to bend toward one another in pairs and unite (Figs. 5 and 6). Fig. 4 is a cross-section of a nucleus at this stage. Thus synapsis takes place in the early part of the growth period soon after the last spermatogonial division. This agrees with Montgomery's ('03) description of the synapsis stage in *Amphibia* and furnishes more detailed evidence of the process.

Up to this period the idiochromosomes have been indistinguishable from the others, but a few very clear sections were obtained which showed at the base of the loops, close to the nuclear membrane, the larger member of the unequal pair (Fig. 5). This suggests the probability that the idiochromosomes retain their characteristic form during the greater part if not all of the synizesis and synapsis stages. All the chromatin has a strong affinity for stains during these periods, and the spireme is most distinct when first formed (Fig. 7), but almost immediately it becomes very faint (Fig. 8). The differential chromosomes still stain deeply, however. The spireme soon breaks up into separate rods, each showing a longitudinal split (Figs. 9, 10), even at this time there seems to be a characteristic form, one chromosome assumes a U-shape, one a triangular form, and one that of a nearly closed ring. These show still more clearly in the later prophase (Fig. 12).

The split disappears (Fig. 11), the chromosomes condense, growing slightly thicker and shorter (Fig. 12), until we get such forms as appear in Fig. 13. The centrosomes are large and distinct in this material, and at this stage two may be seen lying close against the nuclear membrane. In Fig. 13 we see them nearly 180° apart, and as yet no spindle fibers have appeared. Throughout the spireme stage the idiochromosomes are dense spherical bodies. In many cases only the larger member is visible (Figs. 7, 8, 9). In Fig. 11 both are plainly seen. In the later stages while the ordinary chromosomes are undergoing great changes the differential chromosomes remain the same (Figs. 12, 13).

An equatorial plate of the first spermatocyte (Fig. 15) shows eleven chromosomes, and since the spindle fibers are very large, these may often be seen in cross-section attached to the periphery of the chromosomes (Fig. 15). When the material is faintly

stained one chromosome stands out prominently, due to its retention of the stain (Fig. 14). This is the larger member of the differential group, as may be seen in a lateral view of the spindle (Fig. 21). The small idiochromosome fails to show here, due probably to the angle at which the pair is lying; in late prophase (Figs. 17, 22) the pair is easily distinguished. While in numerous metaphases the small chromosome is difficult to discern, there are a few cases in which it is clearly seen (Fig. 18). This unequal pair exhibits no difference in behavior during mitosis, but like the others, and at the same time, with the exception of one which always divides early (Figs. 18, 21), it divides transversely, the large member passing to one pole and the small member to the other (Fig. 25).

We have in this division one of the clearest cases of reduction division possible. In the spermatogonium the chromosomes are V-shaped. During the condensation stage they elongate into loops, at first closely massed together, but later these straighten and unite in synapsis to form longer loops. This is followed by the formation of a continuous spireme (Figs. 7 and 8) which soon breaks up into segments, each split longitudinally (Figs. 9, 10). In the prophase of the first spermatocyte many of the chromosomes are thick rods, which upon examination are seen to be two V's joined end to end, giving in profile a typical E-shape (Fig. 16, *e*). The spindle fiber is attached at the vertex of each V of the bivalent chromosome and in anaphase this element separates at the original place of union, at right angles to the initial longitudinal split (Figs. 18, 20). The V-shaped chromosomes observed in the spermatogonium are thus restored and carried into the second spermatocyte. Exactly the same thing is true in the separation of the components of the cross-shaped chromosome (Figs. 16, *a—d*), and of the ring-shaped one (Fig. 16, *l—o*, and Figs. 22, 23, 24).

There is no rest stage between the two generations of spermatocytes, but almost immediately the massed chromatin in the late anaphase (Fig. 26) separates into chromosomes which begin to arrange themselves in the equatorial plate (Figs. 27, 28, 29). In Fig. 30 we see this in progress, the small chromosome being very distinct. In Figs. 27 and 28, we again see eleven chromosomes

of which one is the small idiochromosome. Having seen that in the first division the large idiochromosome goes to one pole and the small to the other, we should expect to find half the daughter cells containing the large and half the small chromosome of this pair. This is exactly the case. Fig. 29 shows a representative of the cells possessing the large member. This is especially clear in material very much destained, for here as in earlier stages this peculiar chromosome holds the color much longer than the others. In other respects the differential chromosomes are not different, but divide longitudinally with the ordinary chromosomes.

In early anaphase each V shows a distinct split which lies in the plane of the equatorial plate (Fig. 31). That this is the original longitudinal split seen in the early prophase (Figs. 9, 10) there can be no doubt. One chromosome which maintains its identity from the early prophase through the first spermatocyte as a cross is sufficient evidence. In the late prophase and even in metaphase this chromosome (Fig. 16, *a—e*) may be seen to be longitudinally split (Fig. 16, *d*). The line of separation comes at right angles to this split and the univalent chromosomes pass to the poles as V's, each possessing a longitudinal split, though it closes and cannot be seen again until the beginning of the following anaphase. The chromosomes are typically arranged with the vertices attached to the fibers and pointing inward. In division they are drawn apart, at first with the ends of the V's pointing poleward (Figs. 31, 32) but later the vertices turn toward the poles, though disorder often reigns until a comparatively late anaphase (Fig. 33).

In the spermatids there is a great amount of archoplasm, and in the late telophase of the second division it occupies the greater part of the cytoplasmic area (Fig. 36). At a slightly later stage the mass loses entirely its fibrillar structure and, condensed somewhat, lies as a gray sphere (iron hæmatoxylin) against the nucleus (Fig. 38). This is the so-called *nebenkern* of the spermatid. The entire cell now elongates, and with it the axial filament. The archoplasm has assumed the form of a large pennant attached to the nuclear membrane, and the axial fiber runs throughout its length and often beyond (Fig. 39).

A cross-section of the tail at this time shows that the archoplasmic sheath is folding about the axial fiber (Fig. 50, *a*). When the transformation is nearly complete the two cannot be distinguished from each other, the sheath lies close against the filament with a thin layer of cytoplasm outside (Fig. 50, *c*). Often in cross-section the sheath appears to be split (Fig. 50, *b*). Since longitudinal views of the tails reveal nothing of the kind we are left to conjecture that this appearance is due to oblique sections of the trough-shape sheath. The fact that many of these appear together does not alter the explanation, for the spermatozoa and late spermatids lie parallel in large numbers.

There is one peculiar but apparently typical stage in the development of the tail: while the head is yet round, though the chromatin is much condensed, the axial filament has assumed a vacuolated appearance (Fig. 40). This lasts until the head has begun to elongate, and is just over in Fig. 41. This is not to be confused with the rare occurrences of double filaments seen in Fig. 48. These are the products of giant cells, due to a failure of one of the divisions of the spermatocytes or the spermatogonia to complete itself. They consequently have two centrosomes and two axial filaments. Paulmier ('99) found similar conditions in *Anasa*. As in other insects the axial filament in the Coleoptera seems to arise in close relation with the centrosome.

After the vacuolated stage the tail narrows and lengthens much more and we see it in its final form in Fig. 44. All this time the head of the spermatid has been changing. The dense chromatin mass begins to lose its affinity for hæmatoxylin until a deep gray results. A structure is now revealed in the nucleus, not heretofore seen, a small densely staining nucleolus-like body (Fig. 36). The nucleus now widens its circumference and the chromatin condenses around the membrane in the form of a ragged border (Fig. 38). The nucleolus-like formation is not seen at this stage, but the supposition is that it is merely obscured by the dense patches of chromatin, for in the next stage it again appears and occupies a most characteristic position: the chromatin is arranged in a crescent shape and the nucleolus lies in a clear area between the arms of the crescent (Fig. 37). The chromatin becomes diffuse and min-

gles with the karyolymph until a uniform light gray results (iron hæmatoxylin). The nucleolus is unaffected, changing neither in size nor in staining reaction. It makes a change, however, in position about this time, moving from a point opposite the centrosome around the periphery of the nucleus until it often lies very near the centrosome (Fig. 49).

The head of the spermatid lengthens and becomes vacuolated (Fig. 41), but as the elongation increases the vacuoles disappear and the deeply staining nucleolus-like body is apparent, lying closely against the nuclear membrane (Fig. 45, y). Very soon this single body breaks up into two, three or four parts and later, it disappears (Figs. 46 and 47). The head condenses, lengthens, stains intensely (Fig. 42), and in its final form is spirally twisted, resembling the *Bacterium spirillum* (Figs. 43, 44).

It is impossible at the present time to say just what may be the function of this peculiar, deeply-staining spermatid element. It suggests the accessory of Orthoptera which can be seen in one-half the spermatids (McClung, '99, '00, '02a), and at first it seemed probable that it might be one of the idiochromosomes in the beetle as it, in one stage, is about the size of the larger member of the pair. Instead of being found in half the cells, however, it is without doubt in all, so that this explanation was relinquished. It is characteristic of the spermatids of all the beetles thus far studied and of some of the Orthoptera.

## 2. *Coptocyclus guttata*

In external appearance *Coptocyclus guttata* is very different from *C. aurichalcea*, but in their germ cells there are many points of resemblance. *Guttata* has the smaller number of chromosomes, eighteen in the spermatogonia instead of twenty-two, but the size and form are much the same in the two species (Figs. 1 and 51). The unequal pair is also present here.

The suggestion that synapsis takes place immediately after the condensation stage in *C. aurichalcea* is strongly confirmed by *guttata*. In the condensation or synizesis stage, the chromatin is in

the form of loops with the ends against the side of the nuclear membrane (Fig. 52). This point Montgomery calls the distal pole of the nucleus and the opposite point on the nuclear membrane, the central pole. The process is the same as in *aurichalcea*; the loops straighten, thrust the free ends toward the central pole (Fig. 53), bend toward each other in pairs and unite end to end (Fig. 54). The end of the chromatin thread either bears an enlargement or stains more deeply in cross-section for there is at this point the appearance of a deeply staining bead. This dark spot thus conveniently marks the place of union in the bivalent chromosomes, and we are led to the conclusion that the short loops first observed in the synizesis stage are univalent chromosomes. The idiochromosome pair is very distinct at the base of the loops as shown in Fig. 52.

The growth stages are not unlike those of *C. aurichalcea*. A spireme (Fig. 55) is formed which varies in its staining reactions exactly as in *C. aurichalcea*. The thread finally breaks up into bivalent elements which stain deeply and exhibit a longitudinal split, and at last condense into forms which they retain during the first division. In Fig. 57 are seen two bivalent chromosomes assuming the form of rings, and in Fig. 58 these have closed together. This figure also exhibits crosses which later change to the form found in *aurichalcea*. Most of the chromosomes of this species are dumb-bell-shaped in the first spermatocyte mitosis and of the usual V-shape in the second. The idiochromosomes maintain their spherical form throughout all the stages.

An equatorial plate of the first maturation division shows nine chromosomes arranged usually in the order seen in Fig. 60, with seven in a circle about the other two. All nine chromosomes may be seen in lateral view in Fig. 59, where they are just coming into the spindle. The dumb-bell shape of four is here quite evident and the unequal pair is conspicuous. Three of the others appear as straight rods and the other one bent in V-form.

During metaphase the chromosomes arrange themselves with their long axes parallel with the axis of the spindle, and later they divide at right angles to their length. In other words, they exhibit qualitative division, bivalent chromosomes separating at the point

of union made during synapsis. As a result the small chromosome of the idiochromosome pair goes to one pole while the large member goes to the other (Fig. 63). The equatorial plates of the second division confirm this, half possessing nine V-shaped chromosomes of approximately equal size (Fig. 71), the other half having eight large and one small chromosome (Fig. 65). Fig. 66 shows the second maturation spindle with this small chromosome in metaphase, and in Fig. 64 it is seen dividing.

Here, then, as in *C. aurichalcea* and *Tenebrio molitor* (Stevens, '05) half the spermatids will possess the small idiochromosome, and half the large.

The transformation of the spermatid is essentially the same as in *aurichalcea*. The deeply staining nucleolus-like body is present but no clue to its function or nature is given. The ripe spermatozoa are not spirally twisted but resemble that stage of *aurichalcea* seen in Fig. 42.

Owing to lack of material it was impossible to study the dividing somatic cells of the male and female forms. That they would exhibit the same conditions found repeatedly in other beetles there is no doubt. By permission I have reproduced four drawings from Dr. Stevens' originals, which show the chromosome of (Fig. 67) a somatic cell from the digestive tract of a male pupa of *Tenebrio molitor*, and (Fig. 68), a female somatic cell of the same form found in the egg follicle; (Fig. 69) a female somatic cell of *Trirhabda virgata* from the egg follicle, and (Fig. 70) a male somatic cell taken from the larval body of the same species.

Here, as in other forms that Dr. Stevens has investigated, the small idiochromosome goes to the male and the large one to the female.

#### SUMMARY OF OBSERVATIONS

##### *Coptocycla aurichalcea*

1 The spermatogonial number of chromosomes in *Coptocycla aurichalcea* is twenty-two, twenty-one of which are V-shaped and one very small one, spherical in form.

2 Synapsis takes place in a manner, so far not described but also observed in certain beetles now being investigated by Dr. N. M. Stevens. The loops seen in the synizesis stage, which represent individual chromosomes, straighten and unite in pairs by the free ends which are pushed up into the nuclear space. Pseudo-reduction, therefore, occurs just after synizesis and just before the formation of the spireme.

3 The first maturation mitosis is a transverse, reducing division, the second longitudinal, occurring along a lengthwise split formed early in the prophase.

4 There is present a typical pair of idiochromosomes [according to Wilson's definition ('05 and '06)] which, with the others, divides qualitatively in the first division and quantitatively in the second, the small member, therefore, going to one-half the spermatozoa and the large member to the other half.

5 The chromosomes show marked individuality from the beginning of the prophase, one having the form of a ring, two of a cross, several the shape of an E, and finally the unequal pair.

### *Coptocycla guttata*

1 The spermatogonial number of chromosomes is eighteen, seventeen large V-shaped chromosomes and one which is small and spherical.

2 All other observations on this species confirm those on aurichalcea.

## GENERAL DISCUSSION

### *Individuality of the Chromosomes*

Convincing results have been published in regard to the individuality of the chromosomes by Boveri ('02), who found a difference in their function; by Sutton ('02), who found a difference in size; and by Baumgartner ('04), and others, who have discovered a difference of form.

The idiochromosomes indicate so clearly a difference in function as well as in size that it is unnecessary to go into detail on this

point. The ordinary chromosomes also confirm the size difference; though no careful measurements were made, it is obvious that such differences exist at least in most cases (Fig. 19). However, the difference in form is most evident in *Coptocyclus aurichalcea*, and furnishes another strong support for the doctrine of the individuality of the chromosomes.

Of the eleven bivalent chromosomes several possess forms characteristic enough to mark them as the same in different generations; there are two or three crosses, several E's, a ring and an unequal pair (Fig. 16). There is, doubtless, a size difference that separates those of a group though this is not as obvious in some cases as in others. The ring form occurs only once in this species, but twice in *guttata* and maintains its identity of shape from an early prophase, being much more expanded then, however, than later. It is formed by the V-chromosomes of the spermatogonium uniting by both ends, as is plainly shown in Fig. 16, *l*, where two ends are not firmly joined. In a late metaphase the bivalent pair seems to elongate slightly; this closes the opening and gives the appearance seen in Fig. 23. In profile the ring assumes an oval form, with the central opening much smaller (Fig. 16, *n*), but this is distinguished from the actually elongated phase by the length (Fig. 23).

#### *Mendel's Law*

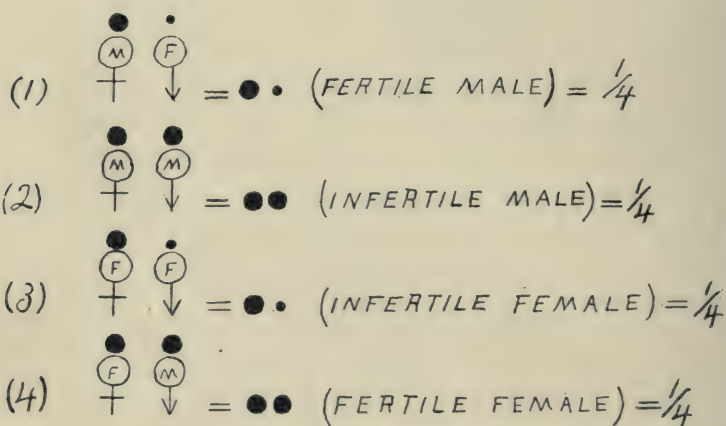
Assuming that the idiochromosomes are sex-chromosomes, or represent the sex-characters, they furnish excellent opportunities for speculation on the application of Mendel's law to chromosomes.

If the Mendelian principles of segregation apply to sex, there should be in the second generation 25 per cent males, 50 per cent hybrids, and 25 per cent females. Castle ('03) concludes that there are no individuals pure in regard to the sex-character, but only hybrids are produced. Now to apply this theory to the beetles. We know by actual observation that the male somatic cells possess the small idiochromosomes, and the female somatic cells the large one. Below are given in a schematic representation

two kinds of eggs and two kinds of spermatozoa with the dot above to indicate the idiochromosome and the letter to show the sex character.



There are four possible combinations here:



Now by actual examination of the male and female somatic cells we know that no such combinations as (2) and (3) exist. Our observations, therefore, strongly support the theory of selective fertilization, only gametes bearing opposite sex-characters fusing.

While the beetle furnishes no explanation why femaleness as a character often dominates (Castle '03) yet its chromosomes indicate why it is recessive a part of the time in these insects. In the 50 per cent of spermatozoa that carry the female character in the form of the small idiochromosome, this is overpowered by the much larger male idiochromosome in the eggs with which they unite. When the chance is even, *i. e.*, when the idiochromosomes are equal in size, then femaleness invariably dominates.

*The Idiochromosomes*

But two groups of animals thus far investigated are known to possess the idiochromosomes; these are the Hemiptera and the Coleoptera. It is probable that they exist in other forms and have been wrongly classified as was the case with Paulmier's ('99) work on *Anasa*, and Montgomery's ('01) on *Cœnus delius* and *Euschistus tristigmus*. Paulmier failed to distinguish between the accessory and the microchromosomes, while Montgomery saw the small idiochromosomes in the resting stages and the different number of chromosomes in different cysts, but misinterpreted them.

To Wilson is due the correct interpretation of these idiochromosomes in the Hemiptera heteroptera, as a distinct pair of chromosomes with a definite and characteristic behavior in the different generations.

Slightly earlier than this and independently Dr. Stevens working on one of the Coleoptera, *Tenebrio molitor*, found an unequal pair of chromosomes, which proves upon comparison to be essentially the same as those of the Hemiptera.

In details of behavior, however, these chromosomes differ markedly in the two groups. As in *Lygæus* and *Cœnus* (Wilson, '05) the idiochromosomes of *Coptocyclus* maintain their identity throughout the growth period. They are first distinguished in the synizesis stage as a compact, spheroidal body at the base of the chromatin loops (Fig. 52). The small member has not been observed at this stage, but it is doubtless present and is obscured by the larger of the idiochromosome pair. As this pair appears in the spheroidal form, so early in the growth stage, it is a question whether it ever assumes the form of loops with the other chromosomes.

In *Lygæus* in the synizesis stage the larger differential chromosome is elongated and bent U-shape. In the post-synaptic phase instead of lengthening and splitting longitudinally as in *Lygæus*, both of the idiochromosomes of *Coptocyclus* maintain their compact, rounded form. In early prophase these chromosomes in Hemiptera exhibit a bipartite structure and sometimes remain separated while the ordinary chromosomes have fused in synapsis.

In *Coptocycla* the unequal pair has fused, but neither member shows any tendency toward being bipartite. In fact, at no time is there a hint of a longitudinal split, unless the vacuolated appearance of the large idiochromosome in the early growth stage may be considered as such.

The later behavior of the idiochromosomes is markedly different in the two groups. In the Hemiptera they remain separated and univalent in the first maturation mitosis, but at the close of this division their products conjugate to form a dyad, which in all but one form, *Nezara*, is asymmetrical. In the Coleoptera the behavior during mitosis is exactly like that of the ordinary chromosomes; having united in the synapsis stage to form a bivalent, they divide transversely in the first mitosis and longitudinally in the second. The result is, of course, the same in both cases; the spermatids are of two kinds as regards the idiochromosomes, half possessing the small and half the large one. The distribution of these chromosomes to the somatic cells of the two sexes is also the same in the two groups; the male cells contain the smaller, the females cells the larger idiochromosome.

#### *Sex-determination*

The observations recorded in the present paper add nothing new to the subject of sex-determination, and their chief value consists in their confirmation of the very suggestive work on *Tenebrio molitor* (Stevens, '05).

Since McClung ('00) advanced his theory that the accessory chromosome of the Orthoptera is a sex-determinant, numerous investigators have sought evidence for or against it. The first in its favor was that of Sutton ('02), who found the odd chromosome present in the male somatic cells and absent in the female cells of *Brachystola magna* (23 in ♂ cells, 22 in ♀ cells). Since that time, however, Wilson has found the reverse true for Hemiptera, *i. e.*, the additional chromosome in the female somatic cells (*Anasa* 21 ♂, 22 ♀), and this leads him to question Sutton's count for the Orthoptera. Wilson suggests that the accessory chromosome of *Anasa*, *Protenor* and the Orthoptera is the homologue of the larger

member of the idiochromosome group found in *Cœnus*, *Lygæus* and certain other Hemiptera, and its missing mate is the homologue of the small idiochromosome. He thus supports the view of Paulmier and Montgomery in regard to degenerating chromosomes.

Knowing from his observations on *Anasa* that the accessory goes to the female in the Hemiptera, he, therefore, conjectured that the larger of the idiochromosomes would be found in the female somatic cells, and the small one in the male somatic cells, and he later found positive proof of this.

As stated before Dr. Stevens while investigating a form of beetle, *Tenebrio molitor*, found that an unequal pair of chromosomes is present, the large one of which goes to the female somatic cells, and the small one to the male somatic cells. Since then she has confirmed this in other species of Coleoptera, so that at last there seems to be much indisputable evidence for the chromosome-sex-determinant theory.

The question arises, what is to be done with such forms as *Thermopsis* (Stevens, '05) and *Banasa* (Wilson, '05). In the former there are no chromosomes which have any external peculiarity that would mark them as sex-determinants; in the latter there are two sets of chromosomes that we interpret as sex-determinants when they appear separately in other forms.

The solution for *Thermopsis* is perhaps less difficult than for *Banasa*; one pair of the chromosomes may bear the sex-character although there are no external differences. This does not conflict with present views. In *Banasa*, the situation is different. Here we have a pair of idiochromosomes as well as a typical accessory. In other cases Wilson has suggested that the large idiochromosome is the homologue of the accessory inasmuch as both are members of pairs, one member of which is degenerating or has already disappeared. This seems plausible and if taken from the standpoint of degeneration only, there is nothing conflicting in the fact that two pairs of chromosomes even in the same cell are undergoing the change. In fact, as Wilson suggests, it adds weight to the idea of degenerating chromatin, for we have no reason to suppose that degeneration is necessarily limited to one pair.

However, taken from the standpoint of sex-determination it seems to offer serious difficulties. While we are perhaps not justified in assuming that the sex character is confined to one chromosome, or to one pair of chromosomes, all recent observations point that way; then on *a priori* grounds it seems improbable that the same function should be assigned to two pairs of chromosomes in the same cell. With the numerous characters to be transmitted it would seem more likely that one pair of chromosomes must be the bearer of many qualities.

In *Banasa* there are four classes of spermatozoa which contain:

- (1) the small idiochromosome + the accessory;
- (2) the large idiochromosome + the accessory;
- (3) the small idiochromosome — the accessory;
- (4) the large idiochromosome — the accessory.

If the chromosome relations in the male somatic cells of *Banasa* correspond to those in *Lygæus* and *Anasa*, only one of these classes of spermatozoa (3) can be used. Any combination of (1), (2), (4) would refute the idea of homology of these two types of chromosomes. The same thing is true as to the production of females; but one kind of spermatozoon (2) can be functional.

This means that three-fourths of the spermatozoa are functionless as regards production of males, three-fourths as regards production of females and one-half absolutely functionless. Such a condition seems most improbable, but, of course, the only way of determining it is to study the male and female somatic cells of *Banasa*. This promises interesting results as it will either refute the homology theory (for this form, at least) or reveal new facts in regard to selective fertilization and a functional and non-functional condition of spermatozoa.

While Castle believes there is no hard and fast rule for dominance in the sex character in diœcious individuals, it seems that in the beetles we have a clear case of female dominance. In cells where the male and female sex chromosomes are equal in size, femaleness invariably dominates. Where they are unequal (the female small, as in Coleoptera and some Hemiptera, or entirely missing as in Orthoptera) then the female character is visibly

recessive, due merely to its reduced strength or entire disappearance in the male.

The results pointed out for *Tenebrio* and confirmed in this paper are briefly as follows: an unequal pair of chromosomes is present which, we have strong evidence for believing, transmit or determine the character of sex. The fact that the small one invariably occurs in the male somatic cells and the large one in the female somatic cells seems on first thought to mean that the small one carries maleness and the large one femaleness. But Wilson's thorough analysis of the conditions in Hemiptera ('06) has shown that the larger idiochromosome which alternates between the sexes must bear the male character, while the small idiochromosome, which is confined to the male sex, must represent the recessive form of the female character.

While it has been proved beyond doubt that certain chromosomes are concerned in the determination of sex among insects, a general application of the theory cannot yet be made, but it must for the present be limited to those forms possessing the accessory or the idiochromosomes. Without doubt further investigation will reveal either similar differential chromosomes in all forms, or show something homologous to them. It may not be a difference of size or shape that will distinguish them from the ordinary chromosomes, but probably one of behavior. Even in case the last difference is not found it need not disprove the chromosome-sex-determinant theory, for the sex character doubtless can be carried like other pairs of antagonistic characters without affecting the chromosome visibly. The eggs of comparatively few forms have been investigated, and it is possible that a dimorphism of the chromosomes in the matured oocytes may be found in certain groups.

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April 30, 1906

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#### DESCRIPTION OF PLATES

All drawings were made with the aid of a camera lucida. A Zeiss oil-immersion 2 mm. objective and oc. 12 were used throughout, and the drawings have been reduced one-third.

## PLATE I

### *Coptocyclus aurichalcea*

- Fig. 1. Equatorial plate of spermatogonial mitosis, 22 chromosomes.
- Fig. 2. Bouquet-stage or synizesis, showing univalent chromosomes in loop form.
- Fig. 3. Later stage in which loops are straightening.
- Fig. 4. Cross-section of the stage in Fig. 2.
- Fig. 5. Synapsis. Fusion of univalent chromosomes end to end at  $y$ . Large idiochromosome  $x$ , at base of loops.
- Fig. 6. Late synaptic stage, showing the relative lengths of the bivalent elements.
- Fig. 7. Formation of spireme;  $x$ , the large idiochromosome.
- Fig. 8. A slightly later stage; spireme very pale,  $x$  deeply stained.
- Figs. 9, 10. Very early prophase, showing the longitudinal split in the chromosomes, idiochromosome excepted.
- Fig. 11. Still later prophase. Both members of the unequal pair visible ( $x$ ).
- Figs. 12, 13. Prophase in which the chromosomes are contracting into their characteristic forms.
- Fig. 14. An equatorial plate of the first division showing nine of the eleven chromosomes. The idiochromosomes retain the stain much longer than the others.
- Fig. 15. An equatorial plate of the first mitosis with the full number, eleven chromosomes, present. The large linen fibers are seen attached to the chromosomes.
- Fig. 16. Three types of chromosomes, found in *C. aurichalcea*, from various points of view,  $a-e$ , the cross-shaped chromosome;  $a$ , between a front and side view;  $b$ , front view when the longitudinal split is closed;  $c$  and  $e$ , profile;  $d$ , front view showing split,  $f-h$ , the E-shaped chromosome  $f$ , front view;  $g$ , profile, bivalent chromosome dividing;  $h$ , profile;  $i-o$ , ring-shaped chromosomes;  $i$ , the two univalent chromosomes not well fused;  $m$ , probably later stage in which union is more complete;  $n$ , ring closed and seen in profile;  $o$ , central opening growing smaller.
- Fig. 17. Late prophase of first spermatocyte; chromosomes coming into equatorial plate;  $x$ , the idiochromosome pair.
- Fig. 18. Equatorial plate formed and one chromosome divided ahead of the others.
- Fig. 19. Shows the size difference in some of the chromosomes.
- Fig. 20. Anaphase of the first division, showing distinctly the form of the univalent chromosomes, and the manner of separation.
- Fig. 21. Side view, first spindle, showing the densely staining idiochromosome when the others are very pale.
- Figs. 22-24. Different views of the ring chromosome.
- Fig. 25. Idiochromosomes dividing in first mitosis;  $l$ , large chromosome;  $s$ , small chromosome.
- Fig. 26. Late anaphase of first spermatocyte.
- Figs. 27, 28. Equatorial plates of second mitosis, showing 10 large and one small ( $s$ ) chromosome.
- Fig. 29. Equatorial plate of same mitosis, showing 11 large chromosomes,  $l$ , the large idiochromosome.
- Fig. 30. Prophase of second mitosis, showing 10 large chromosomes and the small idiochromosome.
- Fig. 31. Anaphase in which the large idiochromosome is dividing.



## PLATE II

### *Coptocycla aurichalcea*

Fig. 32. Anaphase of second maturation division, showing the position assumed by the chromosomes when first separated.

Fig. 33. Late anaphase; small idiochromosomes dividing.

Fig. 34. Late anaphase; unusual case in which the form of the chromosomes is not obscured by massing.

Fig. 35. The usual late anaphase.

Fig. 36. Very early spermatid showing a great amount of archoplasm.

Fig. 37. Nucleus at a slightly later stage, showing the peculiar nucleolus-like body.

Fig. 38. Archoplasmic substance in the form of a sphere applied to nuclear membrane.

Fig. 39. Archoplasm elongated into a pennant form with axial filament running throughout.

Fig. 40. Vacuolated stage in formation of the tail.


Fig. 41. Vacuolated appearance of head *c*, centrosome.

Figs. 42-44. Later stage in the transformation of the head. Ripe spermatozoa (44) possess a spirally twisted head.

Figs. 45-47. Later behavior of the nucleolus-like body.

Fig. 48. Giant spermatozoa, with two centrosomes and two axial filaments.

Fig. 49. Shows the migration of the nucleolus-like body.

Fig. 50. Cross-sections of the tail of the spermatid, *a-b*, at about the stages shown in Fig. 41; *c*, in Fig. 44. 

### *Coptocycla guttata*

Fig. 51. Equatorial plate of spermatogonial mitosis; 18 chromosomes.

Fig. 52. Synizesis stage showing univalent chromosomes in loop form. Large idiochromosome at base of loops.

Fig. 53. Later stage in which loops are straightening and bending toward each other.

Fig. 54. Synapsis. The dark bead at the highest part of loop marks point of union.

Fig. 55. Spireme stage; chromatin very pale with exception of unequal pair (*x*).

Fig. 56. Same stage, showing small idiochromosome somewhat removed from the large member and connected with it by a chromatin strand.

Figs. 57, 58. Formation of ring and cross-shaped chromosomes.

Fig. 59. Late prophase in which all nine chromosomes are seen from a side view. *x*, the idiochromosome pair.

Fig. 60. Equatorial plate of the first maturation mitosis; 9 chromosomes.

Figs. 61, 62. Prophase and metaphase, respectively, showing the idiochromosome pair (*x*).

Fig. 63. Anaphase of first mitosis; the idiochromosome pair, dividing qualitatively. *s*, small idiochromosome; *l*, large.

Fig. 64. Very early anaphase of second mitosis, showing division of the small (*s*) idiochromosome.

Fig. 65. An equatorial plate of the second spermatocyte in which the small idiochromosome (*s*) is present.

Fig. 66. Metaphase of second spermatocyte. The small idiochromosome (*s*) undivided.

Fig. 67. Equatorial plate from dividing somatic cell of male pupa (*Tenebrio molitor*), showing nineteen large and one small chromosome.

Fig. 68. Equatorial plate of a dividing cell of follicle of a young egg (*Tenebrio molitor*), showing twenty large chromosomes.

Fig. 69. Equatorial plate of a dividing follicle cell of a young egg (*Trirhabda virgata*), showing twenty-eight large chromosomes.

Fig. 70. Equatorial plate from the somatic cells of a male larva (*Trirhabda virgata*), showing twenty-seven large and one small chromosome.

Fig. 71. An equatorial plate of same spermatocyte in which all nine chromosomes are of approximately equal size.





# STUDIES ON THE DEVELOPMENT OF THE STARFISH EGG<sup>1</sup>

BY

D. H. TENNENT AND M. J. HOGUE

WITH FIVE PLATES ✓

## INTRODUCTION

The studies made in the preparation of this paper have led to the view that a conjugation of sperm and egg chromosomes takes place soon after fertilization in eggs which have been treated with  $\text{CO}_2$  and subsequently fertilized. That this process takes place in normally fertilized eggs is suggested by the similarity in shape of chromosomes in eggs of these two classes, but no detailed study of the processes occurring in normally fertilized eggs has been made.

This interpretation is made with caution and it is recognized that its truth can be determined only by a reinvestigation of the processes occurring in normally fertilized eggs, or better, by a careful study of the changes taking place during the formation of the germ cells.

Inasmuch as most of the accounts of the cytological processes occurring during artificial parthenogenesis have been based on experiments performed on eggs which had given off their polar bodies while the egg was still within the ovary, it seemed that further observations on an egg which might be subjected, either before or after the extrusion of the polar bodies, to influences capable of causing parthenogenetic development might be of interest.

<sup>1</sup>The experimental work on starfish eggs, described in this paper, was done by the senior author in the summer of 1905, at the Marine Biological Laboratory, at Woods Hole. Some of the material then obtained has been studied during the year 1905-06 in the Biological Laboratory of Bryn Mawr College. Miss Hogue has studied the eggs developing as a result of treatment with  $\text{CO}_2$  sea-water and has written the account of the nuclear changes, seen in sections of these eggs, given in section 1 of this paper. For the remainder of the paper the senior author is responsible.

The eggs of the starfish (*Asterias forbesii*) lend themselves to such observations. These eggs, as is well known, if ripe when removed from the ovaries and allowed to remain in sea-water, soon mature, and further, these eggs, like the eggs of other Echinoderms and unlike the eggs of Molluscs and Annelids, may complete their maturation phenomena before the entrance of the spermatozoon.

Delage's accounts ('02, '04) of the use of  $\text{CO}_2$  in the treatment of the eggs of *Asterias glacialis* naturally suggested that his convenient method might be found useful in experiments on the eggs of *Asterias forbesii*.

Delage ('02) made use of a siphon in which sea-water was charged with  $\text{CO}_2$  by means of sparklet bulbs. His best results were obtained with eggs which were subjected when in the "stade critique" to the action of charged water for an hour, after which the eggs were removed to ordinary sea-water.

During the work of which this paper is an account it was soon recognized that the duration of immersion mentioned by Delage as most favorable for the eggs of *Asterias glacialis* is much too long for the eggs of *Asterias forbesii*. The eggs of this starfish when allowed to remain in  $\text{CO}_2$  sea-water<sup>1</sup> for more than half an hour were apparently killed, as they disintegrated without undergoing development.

Eggs in various stages of maturity were subjected to the action of  $\text{CO}_2$  sea-water for varying periods of time. The details of these trials are unnecessary. It is sufficient to say that the egg of *Asterias forbesii*, like the egg of *Asterias glacialis*, is in its most favorable condition during the time that elapses between the extrusion of the first and of the second polar body. With the eggs of *Asterias forbesii* it was found that uniformly the best results were obtained when the eggs were subjected to the action of the  $\text{CO}_2$  sea-water immediately after the appearance of the first polar body as a protrusion from the surface of the egg.

The best length of time of immersion was about five minutes. Good results were obtained by the immersion of the eggs in  $\text{CO}_2$

<sup>1</sup>Throughout this paper the term " $\text{CO}_2$  sea-water" will be used instead of the longer phrase "sea-water charged with  $\text{CO}_2$ ."

sea-water for from three to ten minutes, but five or six minutes gave uniformly the best results. However, in one lot of eggs in which the time was ten minutes, fully 95 per cent of the eggs segmented regularly and gave rise to normal swimming embryos which were kept alive and under observation for more than a month.

It seemed that the best results were obtained when the sea-water was charged and allowed to stand in the siphon for from ten to twelve hours before using.

### *Method of Treatment*

The eggs were shaken from the ovaries into large dishes of sea-water, in which they were allowed to remain until the first polar body had made its appearance. They were then drawn with a pipette from the bottom of the dish in which they had partially matured and transferred gently and with as little water as possible to finger-bowls. The CO<sub>2</sub> sea-water was then run slowly into the finger-bowl until the bowl was about half-filled. During the whole operation, care was taken to avoid violent agitation of the eggs.

The eggs settled to the bottom of the bowls in about three minutes. At the end of the desired period of immersion, the CO<sub>2</sub> sea-water was withdrawn and replaced by ordinary sea-water, this in turn being changed for fresh sea-water as soon as the eggs had again settled.

The method is convenient in its application and for use with the starfish egg offers an ideal reagent.

### *The Scope of the Investigations*

Since the method is so sure in its action, and since many of the developmental processes reproduce so faithfully the processes occurring in the normally fertilized egg, it seemed that we might have in the starfish egg treated with CO<sub>2</sub> the means of imitating the processes of normal parthenogenesis occurring among rotifers, crustaceans and insects.

Delage ('01) as a conclusion from experiments in which the eggs of *Asterias glacialis* were subjected while in the critical stage (in this case the time when the germinal vesicle loses its membrane and prepares for the emission of the polar globules),

to the action of a solution of KCl, expressed the idea that artificially parthenogenetic eggs, like those in which parthenogenesis occurs naturally, emit but one polar body, and that the agent producing parthenogenetic development acts by the inhibition of the formation of the second polar body, the second polar body playing the role of a spermatozoon.

Delage in 1902 found that eggs submitted to the action of  $\text{CO}_2$ , during the time between the disappearance of the nuclear membrane immediately preceding maturation and of the return of the nucleus to the resting condition ordinarily preceding fertilization, developed independently of the polar globules. Parthenogenesis ('02, p. 231) resulted whether the egg had given off neither, or one, or both of the polar globules.

Subsequently ('04), by a somewhat more complicated method of treatment, he was able to produce parthenogenetic development, using  $\text{CO}_2$  as a reagent, in sea urchin eggs in which he knew that both polar bodies had been given off.

In my work on the eggs of *Asterias forbesii* it became apparent that although the second polar body could not be seen in many of the eggs, it made its appearance in many, and that the eggs developed into swimming embryos in both cases. It was found later that the second polar body was formed in every case, although it might remain within the egg membrane in a cup-shaped depression in the surface of the cytoplasm.

But, although both polar bodies were extruded, thus removing the possibility of imitating exactly the processes of normal parthenogenesis, there was as yet no evidence that part of the chromatin normally extruded in the second polar body was not retained within the egg and that this might later assume the functions of the sperm nucleus.

In consequence of this possibility, it seemed that if it were possible to fertilize the egg after its subjection to the action of  $\text{CO}_2$ , this retained chromatin might be rejected or at least some series of changes might be caused that would be of interest when compared with the normal maturation and fertilization stages and with those occurring in the egg which had been induced to develop by treatment with  $\text{CO}_2$  sea-water.

This idea involved the question of the comparative effectiveness of the  $\text{CO}_2$  solution and of the starfish spermatozoon on the egg of the starfish.

After determining that it was possible to fertilize eggs after they had been immersed in  $\text{CO}_2$  sea-water, the question quite naturally arose: What will be the result of treating fertilized eggs with  $\text{CO}_2$  sea-water?

The results of the investigations may be discussed to the best advantage in three sections.

Section 1 embodies the observations on living eggs after their treatment with  $\text{CO}_2$  sea-water and the data obtained from a study of the sections of the eggs so treated.

Section 2 contains the data obtained from a study of eggs which were treated with  $\text{CO}_2$  and subsequently fertilized.

Section 3 gives the result of the examination of eggs that were fertilized and subsequently subjected to the action of  $\text{CO}_2$  sea-water.

#### I. UNFERTILIZED EGGS TREATED WITH $\text{CO}_2$ SEA-WATER

##### *a. Observations on the Living Eggs*

The time required for maturation varies, as is well known, with the condition of the egg, the surrounding temperature, etc., so that any facts which were observed as to variations of this kind are without value in this connection, but it is of importance to notice the influence of  $\text{CO}_2$  sea-water in delaying the completion of maturation.

In one lot of eggs the first polar body was given off one hour and ten minutes after the removal of the eggs from the ovary. In the eggs of this lot treated with  $\text{CO}_2$  sea-water the second polar body appeared one hour and fifteen minutes after the first had been extruded or two hours and twenty-five minutes after removal from the ovary.

In the stock dish a quantity of the same lot of eggs were allowed to complete their maturation undisturbed and the second polar body appeared thirty-five minutes after the first or one hour and forty-five minutes after the eggs were removed from the ovary.

The  $\text{CO}_2$  sea-water is thus seen to have delayed the process of maturation forty minutes.

In some lots of eggs, but not in all, a distinct membrane, somewhat thicker than the ordinary fertilization membrane, was formed and pushed out from the surface of the eggs when these were transferred from the  $\text{CO}_2$  sea-water to ordinary sea-water, this activity recalling the facts observed by Lefevre ('05, '06) on eggs of *Thalassema* treated with acid solutions. This membrane carried the first polar body out with it, the second polar body being extruded into the space between the surface of the egg and the membrane.

In the average lot of eggs the series of changes preceding the first cleavage follows very closely, those described by Wilson ('01) as occurring in *Toxopneustes* eggs, the important difference being that in the eggs of *Asterias forbesii* that have received the best degree of treatment with  $\text{CO}_2$  sea-water no cytasters were to be observed. In eggs which had received too prolonged a treatment, numerous cytasters might be seen.

In about three and a half hours after removal from the  $\text{CO}_2$  sea-water the cytoplasm becomes coarser and looser in appearance, apparently becoming more fluid in character in the region of the nucleus. Radiations appear stretching out from the vaguely defined clearer area into the denser cytoplasm. As a result of these changes the nucleus becomes very distinct.

These primary radiations then become fainter and new radiations growing in from the sides of the nucleus are seen, the nuclear membrane breaks down and the definite mitotic figure is seen to be forming, its centers gradually enlarging as the figure becomes definitely established. The division of the egg into two cells is completed in about four hours after removal from the  $\text{CO}_2$  sea-water. On one or two occasions, the temperature being low, the first division was completed only after five hours.

It is of interest to notice that in the eggs of *Asterias glacialis* observed by Delage ('02) segmentation commenced about three hours after removal to the ordinary sea-water, the eggs having remained in the  $\text{CO}_2$  sea-water for an hour, that is, segmentation commenced four hours after the beginning of the treatment, while

in the eggs of *Asterias forbesii*, observed by me, the segmentation began in approximately the same time, about four hours from the beginning of the treatment, although the eggs had remained in the  $\text{CO}_2$  sea-water for but five or six minutes.

*b. A Study of the Nuclear Changes as Seen in Sections*

Delage in his work on *Asterias glacialis* did not make a detailed study of these phenomena. The purpose of his work being finally ('04) to raise the larvæ until they would metamorphose.

Morgan's ('99) observations on the eggs of *Asterias forbesii* when treated with solutions of NaCl, MgCl and KCl ('99, p. 499) although brief, since the eggs were immature, are of interest. He mentions the appearance of areas of cyanoplasm and the fact that in some unfertilized eggs that had been in a solution of magnesium chloride for three hours, stars with delicate rays made their appearance in these areas of cyanoplasm.

Kostanecki's work on the eggs of *Mactra* ('04) is of especial interest in the present connection, since in this egg, which normally does not extrude its polar bodies until after the entrance of the spermatozoan, he traced a series of phenomena analogous to those observed in the starfish egg.

The eggs of *Mactra* were treated with solutions of KCl, NaCl, CaCl or with concentrated sea-water. The most normal development was obtained in eggs which remained in a weak solution of KCl thirty minutes. Here both polar bodies were given off ('04, Fig. 34) and from the chromosomes remaining in the egg a nucleus was formed quite as in the fertilized egg, although it contained but twelve chromosomes, one-half the normal number (twenty-four), as was later shown in the first segmentation spindle.

The polar spindles developed deeper within the cytoplasm and the polar bodies were larger than those of fertilized eggs. In the manner of division of the segmentation nucleus and in the absence of centrosomes and centrosome-like structures in these divisions, the eggs of *Mactra* differ from those of *Asterias forbesii*.

Scott ('06), in his observations on the parthenogenetic development of *Amphitrite* eggs after treatment with salt solutions, shows that the ripeness of the eggs, the strength of the solutions,

etc., were the factors determining whether development would be nearly normal or very abnormal. If the *Amphitrite* eggs are ripe, normal polar bodies are given off in a weak solution of calcium nitrate, although the subsequent segmentation is abnormal. Here, again, little comparison can be made between these eggs and those of *Asterias forbesii*, the most important point of agreement being that both polar bodies are given off in each case.

Lefevre's ('06) work on artificial parthenogenesis in the eggs of *Thalassema* shows many points of agreement with my observations on starfish eggs, the formation of the "fertilization membrane," the extrusion, as a rule, of both polar bodies may be mentioned here and further comparisons reserved for a later mention.

In the present experiment the eggs, after the extrusion of the first polar body, were covered with sea-water charged with carbon dioxide. They remained in this four minutes and were then transferred to ordinary sea-water.

The series consisted of twenty-five stages, the earlier numbers of which were fixed at five minute and the later at ten minute intervals in Boveri's picro-acetic acid.

The eggs were cut in sections 3 microns thick and stained with Heidenhain's iron-hæmatoxylin, long method. Eosin, erythrosin, and Bordeaux red were tried as counter-stains but the iron-hæmatoxylin gave the clearest and best effects. Total mounts were stained by Conklin's hæmatoxylin method.

Throughout the series a few eggs were found with the germinal vesicle intact, which is due to the fact that the nuclear membrane had not begun to fade when the eggs were treated with the CO<sub>2</sub> sea-water, *i. e.*, they were not in Delage's "critical stage."

After the first polar body is given off, a cone of cytoplasm is often seen projecting from the surface of the egg at the side of the first body during the time that the second polar body is forming. This cone frequently persists until the first segmentation spindle is well formed (Figs. 21 and 26) and is occasionally seen in the two-cell stage at the edge of the cleavage plane.

The polar bodies are of the same size as those given off in fer-

tilized eggs, differing in this respect from those observed by Kostanecki in the artificially parthenogenetic eggs of *Mactra*.

The cleavage is normal, dividing the egg into halves and the second forming four equal-sized blastomeres.

Shortly after the extrusion of the first polar body the chromosomes are found lying on the spindle fibers, which have already begun to degenerate at their polar ends (Fig. 1). In a later stage, eighteen chromosomes may be counted (Fig. 2), and with them are seen a few spindle fibers. These fibers disappear and the chromatin is left free in the cytoplasm (Fig. 3), more or less massed together. In the cytoplasm directly beneath the first polar body is a darkly staining region which is not represented in the drawings. It does not take the chromatin stain.

The formation of the second polar body proceeds slowly and the time of the division of the chromosomes varies. In an egg from stage 5 (Fig. 4) the chromosomes, undivided, are still attached to the fibers of the first polar spindle. In stage 11 (Fig. 5) the chromosomes are divided for the second polar body although they are still attached to the fibers of the first polar spindle.

From a study of the preserved material, it seems evident that the second polar spindle is formed tangentially to the surface of the egg (Fig. 6) and that it later revolves until it has taken a radial position (Figs. 7 and 8). The centrosomes, single and double, may be seen clearly in these spindles. (Figs. 6, 7 and 8.)

In the early anaphase of the second polar spindles (Fig. 9) the chromosomes are scattered irregularly over the whole spindle. In the late anaphase (Fig. 10) they are collected in plates at the two poles of the spindle, one or two of the chromosomes being later than the others in taking their positions.

After the second polar body is formed, the chromatin is more or less free in the cytoplasm, some of it lying on the astral rays as though passing down these into the cytoplasm (Fig. 12), and the remainder forming five or six vesicles which fuse to form the female pronucleus. At each division of the nucleus, chromatin is thrown out into the cytoplasm. Occasionally the chromatin is found lying free in the cytoplasm, without a trace of astral radiation or of vesicle formation (Fig. 11).

The newly formed nucleus now moves to the center of the egg and at the same time begins to divide. The walls of the vesicle become indented (Fig. 13), and on the side nearest the surface of the egg two centrosomes appear and move around the nucleus until they lie one at each end of the slightly elongated nucleus. This process differs from that described by Lefevre ('06) for *Thalassema* in which the cleavage asters with their centers arise simultaneously at opposite poles of the egg nucleus.

At the same time the astral fibers are forming in the cytoplasm. This growth apparently begins at the nuclear membrane and extends into the cytoplasm of the egg as described by Wilson ('01) and Morgan ('99) for Echinoderm eggs. Later, these radiations collect at the two ends of the nuclear vesicle whither the centrosomes have migrated.

Following this stage the nuclear membrane breaks down and particles of chromatin, seen in Fig. 15 on the astral rays, pass into the cytoplasm.

As the nuclear membrane disappears (Fig. 16), the conspicuous nucleolus unravels and its chromatin, together with that derived from the chromatin network of the nucleus becomes broken up into short threads, the discharge of chromatin into the cytoplasm continuing meantime. The study of the sections has not shown whether all of the chromosomes of the equatorial plate are derived from the nucleolus. The spindle fibers grow into the nucleus and may be seen stretching from the poles to the center of the nucleus.

At this stage the nuclear sap has been poured out into the cytoplasm and a difference in staining reaction of the areas indicated by the dotted line may be seen.

Figs. 14 and 17 represent later stages of the first segmentation spindle.

In the late anaphase the chromosomes become enclosed in vesicles (Fig. 18), which fuse to form the daughter nuclei. The nuclear membrane forms as the astral rays are disappearing. During this process chromatin rejection continues, the rejected chromatin being seen enclosed in little vesicles lying in the cytoplasm (Fig. 22).

After the daughter nuclei are formed they move apart and the constriction appears which divides the egg into halves. It is interesting to note that the cytoplasm does not begin to divide until the daughter nuclei are completely formed. Figs. 23 and 25 represent the spindle and equatorial plate of the second segmentation.

Mathews ('95) was unable to find centrosomes in the segmentation spindles of *Asterias forbesii*. In the material from which these sections were made two kinds of centrosomes were found; one granular, containing several small deeply staining bodies (Figs. 17 and 23); the other, sometimes a single body, more often two, from which the radiation extend (Fig. 16). In Fig. 18 the double centrosomes appear in the cytoplasm while the astral rays are disappearing.

It has been impossible to count the chromosomes in the segmentation spindle as they do not take definite form (Fig. 14) until they line up in the equatorial plate, ready for division. Here, again, it was useless to try to count them since they were massed together and of irregular shape. Often part of one chromosome is in one section and part in another. Again, some divide before others and frequently when the majority of the chromosomes have separated, a long chain of chromatic material, as yet unsegmented, extends down the middle of the spindle. A few chromosomes seem to have a characteristic form.

It will be remembered that in the second polar spindle there were eighteen chromosomes (Figs. 1, 2 and 8). From Figs. 9 and 10 it seems evident that at least eighteen chromosomes will be left in the egg after the second polar body is given off. Fig. 21 is drawn from a section showing the two polar bodies at the surface and the equatorial plate at the center of the egg. This is shown again drawn under higher magnification in Fig. 20. While it is impossible to count the number of chromosomes exactly, it is evident that there are at least eighteen here. Another egg (Fig. 24) shows the two polar bodies at the surface and the equatorial plate at the center, with the spindle fibers in cross-section. This equatorial plate is shown again in Fig. 19.

In this the chromosomes have divided for the daughter nuclei of the first division.

Delage maintained a doubling in the number of chromosomes in the parthenogenetic eggs of *Strongylocentrotus lividus*. The eggs had given off both polar bodies when treated with the solution and yet later they contained the normal somatic number of chromosomes. He held that at some time, as yet undetermined, the chromosomes divide again and so establish the normal number (autoregulation), these in turn dividing to form the chromosomes of the daughter nuclei. Boveri has since shown that eighteen is not the somatic number of chromosomes for *Strongylocentrotus lividus*, but the reduced number.

In the parthenogenetic eggs of *Mactra*, Kostanecki ('04) found the reduced number of chromosomes in the segmentation spindle when the egg had given off both polar bodies. These eggs did not segment many times.

Boveri ('04) experimenting with the fertilization of nucleated and non-nucleated fragments of sea-urchin eggs, found that when a non-nucleated fragment is fertilized it contains less chromatin; *i. e.*, the nuclei are smaller than the fertilized nucleated fragments. Morgan ('95) performed similar experiments with similar results.

Stevens ('02) while working on the eggs of *Echinus microtuberculatus* which were cut into pieces while in the anaphase of the first division, found that fragments containing a centrosome and a small number of chromosomes may divide five or six times without the chromosomes returning to the constitutional number.

Wilson ('01) showed that in parthenogenetic *Toxopneustes* eggs the number of chromosomes is eighteen, one-half the number occurring in fertilized eggs.

The evidence at present, seems in favor of the permanence of the number of chromosomes occurring in the unfertilized egg which is caused to develop parthenogenetically after two polar bodies have been given off.

Another idea of Delage's, that the presence of two polar bodies might be due to the division of the first polar body, seems incorrect. In *Asterias forbesii* the formation of the second polar body

has been traced and its actual cutting off has been noted. The second polar body is slightly smaller (Figs. 21 and 24) while the first polar body contains the greater amount of chromatin (Fig. 11).

One egg was found in stage 24 (Fig. 24) in which the second polar body had been formed but not extruded from the egg before the nucleus began to divide. Here it is certain that the polar body does not again enter the nucleus. This retention of the second polar body within the egg is not the usual method of procedure but may be accounted for by the tardiness with which this egg began development. There is, of course, a possibility that later the chromatin of this retained polar body might mix with that of the egg nucleus in a manner similar to the behavior of the sperm nucleus in partial fertilization as noted by Boveri.

One cannot but be impressed with the normal procedure of development in the eggs treated by Delage's  $\text{CO}_2$  method. In the thousands of eggs examined in the study of these sections, there were not more than a dozen abnormal structures. (It is to be remembered, of course, that this series which has been studied with greatest detail was selected primarily because of its perfection.)

Two cases of multipolar spindles were observed, one with three poles, the other with four. A few eggs, undivided, contained three nuclei, each with its amphiaster ready for division. There were also two or three eggs which had divided into three blastomeres. The cleavage in the early stages was normal. No cytasters were formed in the eggs.

#### SUMMARY

- 1 Two polar bodies are given off.
- 2 Eighteen single chromosomes are left in the egg after the extrusion of the second polar body. (The number counted varies within slight limits.)
- 3 Centrosomes are present in the polar and segmentation spindles.
- 4 Cleavage is normal though slower than in fertilized eggs.

II. OBSERVATIONS ON EGGS WHICH WERE FIRST TREATED WITH  $\text{CO}_2$  SEA-WATER AND SUBSEQUENTLY FERTILIZED*Description of the Experiments*

Since it was not known whether the eggs were capable of fertilization after removal from the  $\text{CO}_2$  sea-water, some preliminary work was necessary in order to determine, first, whether it was possible to fertilize such eggs, and, second, the optimum time for such fertilization after the eggs had been removed from the  $\text{CO}_2$  sea-water.

A set of eggs was subjected immediately after the appearance of the first polar body, to the action of  $\text{CO}_2$  sea-water for six minutes and then transferred to a large dish of sea-water and used as stock from which at five minute intervals during a period of two hours, eggs were taken and treated with active sperm.

A series of these eggs (24 stages in all), each stage taken ten minutes after the addition of the sperm, was fixed for sectioning and the remainder of each stage were allowed to continue their development. The study of these sections is now but partially completed.

Some data of this work may be of interest. I give brief notes of observations on the stock and four stages from the twenty-four which were under observation.

Stock—Eggs remained in sea-water for one hour and eleven minutes when the first polar body was seen pushing out from the surface of the majority of the eggs (9:15–10:26).

(1) Eggs subjected to  $\text{CO}_2$  sea-water for 8 minutes (10:26–10:34)

(2) 11:15, still but one polar body.

(3) 11:30, two polar bodies and irregular membrane.

(4) 3:00, ten per cent in two-cell stage.

Stage A—Stock eggs treated with sperm at 10:40 (six minutes after removal from  $\text{CO}_2$  sea-water).

(1) 10:43, fertilization membrane distinct.

(2) 11:35, two polar bodies, one inside, one outside of fertilization membrane.

(3) 12:00, beginning two-cell stage.

(4) 3:00, twenty per cent past eight-cell stage.

Stage C—Stock eggs treated with sperm at 10:50 (sixteen minutes after removal from CO<sub>2</sub> sea-water).

(1) 12:08, beginning two-cell stage.

(2) 3:00, twenty per cent past eight-cell stage.

Stage D—Stock eggs treated with sperm at 10:55 (twenty-one minutes after removal from CO<sub>2</sub> sea-water).

(1) 12:10, some segmented.

(2) 3:00, thirty-five per cent segmented.

Stage H—Stock eggs treated with sperm at 11:15 (forty-one minutes after removal from CO<sub>2</sub> sea-water.)

(1) 12:15, beginning two-cell stage.

(2) 3:00, thirty per cent eight-cell stage.

In A eggs began to divide one hour and twenty minutes after fertilization.

In B eggs began to divide one hour and eighteen minutes after fertilization.

In D eggs began to divide one hour and fifteen minutes after fertilization.

In H eggs began to divide one hour after fertilization.

In this experiment the highest percentage of dividing eggs was obtained from those treated with sperm between twenty and forty-five minutes after removal from CO<sub>2</sub> sea-water. After 11:15 through the four succeeding stages 11:20, 11:25, 11:30, 11:35, the percentage of eggs dividing in approximately one hour after fertilization rapidly diminished, being practically zero at 11:35 (5 minutes after the second polar body had been given off in the CO<sub>2</sub> stock).

In all of the succeeding stages, segmentation began between 2:20 and 2:40 or roughly four hours after treatment with CO<sub>2</sub>.

From these observations it seemed possible to conclude that eggs treated with CO<sub>2</sub> sea-water were capable of fertilization during the hour immediately succeeding removal from CO<sub>2</sub> sea-water. After that time had passed the spermatozoa, although still exceedingly active, were either not able to enter the egg or entering it produced no effect and the eggs proceeded to their parthen-

ogenetic division. Fresh spermatozoa were tried but without avail.

For the sake of comparison, a table showing the percentage of eggs developing from a set allowed to mature for one hour and forty minutes (10:00-11:40), in sea-water (until the extrusion of the first polar body), and then fertilized at the intervals noted, is given. These eggs were not treated with  $\text{CO}_2$  but were simply fertilized with sperm.

Fertilized 11:40, ninety-five per cent segmented.

Fertilized 12:03, ninety-five per cent segmented.

Fertilized 12:25, ninety per cent segmented.

Fertilized 1:40, thirty per cent segmented.

Fertilized 3:00, no eggs segmented.

Fertilized 4:00, no eggs segmented.

In this set of normal eggs a considerable percentage was capable of fertilization for more than two hours after the extrusion of the first polar body.

Comparison of these sets of results, then, show that apparently treatment of the eggs with  $\text{CO}_2$  sea-water shortened the time during which the sperm might be effective.

From the observations mentioned it was thus found that the most normal results were obtained by fertilizing the  $\text{CO}_2$  eggs from twenty to forty minutes after their removal from the  $\text{CO}_2$  sea-water. These eggs when successfully fertilized commenced their segmentation in approximately one hour and thirty minutes after fertilization. The treatment with sperm shortened the time elapsing between the treatment with  $\text{CO}_2$  sea-water and the beginning of segmentation, or, stating it more concretely:

The time usually required for segmentation of  $\text{CO}_2$  eggs was about four hours from the extrusion of the first polar body.

The time required for the beginning of segmentation of  $\text{CO}_2$  eggs subsequently fertilized was about one and one-half hours.

The eggs that had been treated with  $\text{CO}_2$  and subsequently fertilized gained in time, then, two and one-half hours.

In work with other lots it was found that the greatest percentage of fertilized eggs was to be obtained, if the  $\text{CO}_2$  eggs were treated

with sperm twenty or thirty minutes after removal from the CO<sub>2</sub> sea-water.

Several sets of eggs were thus treated and from these, series were fixed which have served as the basis of the cytological study.

It is of considerable interest to notice that the thick vitelline membrane pushed out from the egg, did not prevent the entrance of the spermatozoan, and that in eggs fertilized after the appearance of this membrane, a second membrane, somewhat thinner than the first, was formed, so that the eggs were surrounded by concentric membranes.

In several cases, the actual passage of the spermatozoan through the outer membrane was observed, while in another lot of parthenogenetic eggs which were treated with sperm when in the two- and four-cell stages, the spermatozoa were seen swimming actively between the blastomeres.

The sections of these eggs have not been studied so that it is impossible to state whether or not the spermatozoa entered the blastomeres, or their effect on subsequent developments.

#### *The Study of Sections of the Eggs*

The facts determined by the study of sections of eggs treated with CO<sub>2</sub> sea-water and later fertilized, show that the processes occurring are so like the normal that a detailed description is unnecessary. I shall, therefore, content myself with a description of the figures and later make a comparison between the facts brought out by Miss Hogue in her study of the parthenogenetic development and those determined in my own study of the eggs which have received the double treatment.

In some of the eggs sectioned, the first polar body was in process of formation. Here, there seemed absolute evidence of the longitudinal division of bivalent chromosomes.

Fig. 30 shows a section through the remains of the first polar spindle immediately after the extrusion of the first polar body. In the polar body and in the egg the dumb-bell-shaped bivalent chromosomes are seen (Fig. 31). In some cases a tetrad effect (Fig. 32) due, I believe, to the grouping of the double chromosomes was to be seen.

In some cases the chromosomes of the second polar body remained within the egg (Fig. 33), clustered beneath the first polar body where they apparently degenerated, since in slightly later stages they were seen to be broken into small fragments, and in still later stages a few slightly stained granules were found in this position. There was absolutely no evidence that as chromosomes they took any part in subsequent nuclear transformations.

The best evidence shows that in the second maturation division the bivalent chromosomes are divided to form univalent chromosomes (Figs. 34-37), as has been described by Mathews ('95). The division is transverse. I have been unable to find evidence of the double longitudinal division which has been described by Bryce ('02) for *Echinus esculentus*.

In many cases abnormalities occur (Figs. 38-42). In some cases tripolar spindles are seen, in which event it seems possible that both polar bodies are being formed in the same division.

The chromosomes remaining within the egg soon form five or six vesicles which are drawn together and fuse to form the female pronucleus which lies surrounded by some of the degenerating fibers of the second polar spindles (Figs. 44, 48, 49 and 50). Meantime the sperm nucleus, which has become vesicular, has been moving, accompanied by its aster toward the egg nucleus, the centrosome and aster dividing as the two approach (Figs. 45 and 46), the two nuclei finally fusing to form the segmentation nucleus.

In a very few cases the egg nucleus seems to be provided with an aster of its own (Fig 44). In most cases it seems simply lying among some of the fibers remaining from the second polar spindle.

The segmentation nucleus remains in a resting condition for some time, during which the progression of the asters to opposite sides of the nucleus may be observed. From their inner sides fibers may be seen projecting into the nuclear membrane (Fig. 58). and in slightly later stages these fibers may be seen within the nucleus although the membrane seems intact. The nucleus has meantime become decidedly elongated in outline.

Succeeding this stage the conditions represented in Figs. 59-61 rapidly succeed one another. The nuclear membrane apparently dissolves first at the poles and finally dissolves throughout,

during which process the achromatic figure increases greatly in size.

The chromatic reticulum and the nucleolus break down into coarse threads which in succeeding stages becomes finer, these threads ultimately becoming broken up into short rods. (Figs. 54–56). These rods become rounded (Fig. 57) and during the formation of the equatorial plate are replaced by bivalent structures (Figs. 62–68), which lie with their long axis at right angles to the long axis of the spindle.

All of the observations point to a conjugation of rounded univalent chromosomes to form elongated bivalent chromosomes.

In Figs. 54 and 55 the chromosomes are seen to be rod-like. In Fig. 57 the form has changed, all of the chromosomes having become rounded, a few showing the bivalent form. In Figs. 62 and 63, 66 and 67, it is seen that the bivalent form has become more common while the actual number of chromosomes has diminished, while in Fig. 68, which represents all of the chromosomes of the equatorial plate, it is seen that the number of chromosomes has been still farther reduced and that with the exception of six univalent chromosomes all of the chromosomes show the bivalent form.

These chromosomes retain their bivalent form during the segmentation divisions (Figs. 64 and 65 and 61 and 69). In Figs. 61 and 69 it is seen that some of the chromosomes are still of the univalent form. This was true of all of the segmentation stages examined.

The division of the chromosomes in the segmentation is longitudinal, the chromosomes which are at first placed with their long axes at right angles to the long axis of the spindle becoming pulled out so that their long axes lie parallel to that of the spindle (Figs. 64, 65 and 53).

The daughter nuclei are formed by the fusion of chromosomal vesicles.

Granules of chromatin are thrown out into the cytoplasm from the time of the breaking down of the nuclear membrane preceding segmentation until the formation of the daughter nuclei is completed.

Further reference will be made to some of these changes in the general considerations at the end of this paper.

### III. EGGS FERTILIZED AND SUBSEQUENTLY TREATED WITH CO<sub>2</sub>

In these eggs the sperm was allowed to act for ten minutes and the eggs were then transferred to CO<sub>2</sub> sea-water for five minutes. I give the data of but one lot.

Fertilized eggs placed in CO<sub>2</sub> sea-water (12:28–12:33).

No eggs segmented until 3:40 or three hours and seven minutes from the time of removal from the CO<sub>2</sub> sea-water.

Since the sections of these eggs have not yet been studied it is impossible to say definitely whether the eggs developed as a result of the CO<sub>2</sub> treatment or as a result of fertilization by the sperm.

It is probable that since segmentation began within three hours after the eggs were removed from the CO<sub>2</sub> sea-water the sperm fertilization was effective, in which event the action of the CO<sub>2</sub> sea-water was simply to delay development.

#### SUMMARY

The observations on the behavior of eggs treated with CO<sub>2</sub> may be summarized as follows:

1 Unfertilized eggs subjected to the action of CO<sub>2</sub> sea-water for from 3 to 10 minutes commenced segmentation about four hours after treatment.

2 (a) Unfertilized eggs subjected to the action of CO<sub>2</sub> sea-water for from three to ten minutes and fertilized twenty to thirty minutes after removal from the CO<sub>2</sub> sea-water began segmentation about one hour after fertilization.

(b) Unfertilized eggs subjected to the action of CO<sub>2</sub> sea-water for from three to ten minutes when treated with sperm one hour or more after removal from the CO<sub>2</sub> sea-water segmented four hours after removal from the CO<sub>2</sub> sea-water.

3 Fertilized eggs subjected to the action of CO<sub>2</sub> sea-water segmented three hours after removal from the CO<sub>2</sub> sea-water.

GENERAL CONSIDERATIONS

As to the cause of the parthenogenetic development of eggs treated with  $\text{CO}_2$ , whether it is by reason of a change of osmotic pressure, or because of agitation, or of the exposure to some specific chemical substance, or is due to the presence in the sea-water of new compounds formed in reactions which may take place between the  $\text{CO}_2$  or impurities which may be present in the materials used and substances present in sea-water, this paper has nothing to say.

The simple fact remains that eggs treated with  $\text{CO}_2$  as has been described, segment regularly and develop into embryos which in form and structure cannot be distinguished from embryos obtained from fertilized eggs.

Whether the reagent acts as a stimulus, or a shock, or a poison, the author cannot say. The evidence, however, is sufficient to show that Delage was correct in his view that maturation is arrested temporarily by the action of the  $\text{CO}_2$ .

It is in the comparison of sections of  $\text{CO}_2$  eggs with those of  $\text{CO}_2$  eggs which were subsequently fertilized and with those of normally fertilized eggs that the most interesting facts come to light. All behave in essentially the same manner in the maturation processes, these processes apparently agreeing with those described by Mathews ('05) but which he has not figured in detail. In the material studied, which it is to be remembered must be regarded as having received somewhat artificial treatment from the beginning, the number of chromosomes remaining in the egg, after the extrusion of the second polar body, varies slightly, the number ranging from eighteen to twenty-three, although eighteen seems to be the common number.

It is seen that the maturation processes in both the  $\text{CO}_2$  eggs and in the  $\text{CO}_2$  eggs which were subsequently fertilized, differ remarkably from those observed in compressed eggs by King ('06). Here, although the first polar body may be extruded, the second is retained, all of the retained chromatin going into the formation of one or several vesicles which unite to form the egg nucleus, but "the retention of chromatin that is normally extruded

in the polar bodies does not lead to a parthenogenetic development of the egg." In compressed eggs which were fertilized, great differences in size in the two pronuclei were noted, a phenomenon which does not occur in CO<sub>2</sub> eggs subsequently fertilized. The polyspermy observed in fertilized compressed eggs has been noted in CO<sub>2</sub> eggs subsequently fertilized which did not receive the treatment which results in normal development.

It is worthy of note again that in the CO<sub>2</sub> eggs which were subsequently fertilized and in ordinarily fertilized eggs the chromosomes during the maturation divisions are bivalent (Mathews, double chromosomes) and at the close of the maturation divisions eighteen univalent (Mathews, seventeen [?] single) chromosomes remain within the egg.

It will be noticed that the straight CO<sub>2</sub> treatment evidently has some influence on the maturation processes, for while the number of chromosomes remains the same as in the fertilized eggs, the shape of the chromosomes varies.

The process of reconstruction of the egg nucleus is by the formation and fusion of chromosomal vesicles in eggs of the three classes mentioned.

The appearance of the segmentation nucleus in the CO<sub>2</sub> eggs and in the CO<sub>2</sub> eggs which were subsequently fertilized shows no difference. This nucleus breaks down in the same manner in both cases, the nuclear reticulum becomes threadlike and breaks up into fragments, the mass of chromatic material being the same, so far as the eye can judge, in the one case as in the other.

In neither case can the exact number of fragments be counted, but it is clearly to be seen that they are not to be regarded as individual chromosomes since the numbers counted are more than double those found at any earlier or later stages when the count may be made with reasonable certainty, and since many of these fragments may be seen in various stages of withdrawal into the cytoplasm.

But at the time of the completion of the equatorial plate, fundamental differences between the chromosomes of the CO<sub>2</sub> eggs and the eggs which were subsequently fertilized may be seen. In the one case the chromosomes are irregular and in the other, of a

distinct bivalent form; a form which is preserved during the longitudinal splitting of the chromosomes as it divides to form the daughter nuclei, a form which persists in the later divisions of the egg so far as observed and a form which is again seen in the maturation divisions until the final separation of bivalent into univalent chromosomes during the formation of the second polar body.

These facts, it seems to me, are to be explained only by the suggestion that a conjugation or synapsis of egg chromosomes and sperm chromosomes takes place immediately before the formation of the equatorial plate of the first segmentation spindle.

That this process takes place seems very probable when, after comparing Figs. 19 and 20, representing sections through the equatorial plate in  $\text{CO}_2$  eggs, with Figs. 54, 56 and 57, representing sections through the equatorial plate of  $\text{CO}_2$  + sperm eggs, one follows the series of changes shown in Figs. 62, 63, 66, 67 and 68, in which the rounded chromosomes are shown, some lying free, some drawn closely together in pairs and still others showing the completed bivalent form. This form is retained from the equatorial plate stage (Fig. 68), through the division figures ( Figs. 64, 65, 69, 61) of the  $\text{CO}_2$  + sperm eggs and may be seen in the division figures of the normally fertilized eggs ( Figs. 28 and 29).

As a result of such a conjugation the *number* of chromosomes would remain the same in the parthenogenetic as in the fertilized eggs, with the difference that in the parthenogenetic eggs there remain eighteen univalent chromosomes and in the fertilized eggs eighteen bivalent chromosomes.<sup>1</sup>

<sup>1</sup>During the summer of 1906 the experiments which have been described in this paper were repeated and some further observations made. In this work it was demonstrated satisfactorily that the equatorial plate in the eggs from some individuals contained, within slight variations, thirty-six chromosomes. The Woods Hole starfishes thus show a variation as to the number of chromosomes similar to that pointed out in *Echinus microtuberculatus* by Stevens ('02). In such eggs as in the eggs that have been considered in this paper the number of chromosomes remains the same in both fertilized and in parthenogenetic eggs. A point of disagreement between these eggs and those obtained during the previous summer is that dumb-bell-shaped chromosomes were found in parthenogenetic eggs. Little dependence, therefore, as has been suggested by several authors recently, can be placed upon the shape of the chromosomes as an indication of valency.

It is possible that the objection will be made that the activity of the  $\text{CO}_2$  sea-water is such as to change the shape of the chromosomes in the equatorial plate of the first segmentation spindle, since we have seen that the shape of the chromosomes in the maturation divisions was thus influenced.

In replying to such an objection, it can only be pointed out that the number of chromosomes, although these chromosomes vary in size, shape, etc., remains apparently the same in both the  $\text{CO}_2$  eggs and in the  $\text{CO}_2$  eggs which were subsequently fertilized, and this in spite of the fact that we should expect that the number in the fertilized egg to be doubled.

An additional point of interest comes out in the comparison of these eggs, and this in respect to the cleavage asters. The cleavage asters with their centers have the same appearance in both the  $\text{CO}_2$  eggs and in the  $\text{CO}_2$  eggs which were subsequently fertilized. In eggs which were fertilized at the optimum time these were the only asters formed, while in eggs fertilized later than this time many cytasters and additional sperm asters were formed, the subsequent divisions being exceedingly abnormal. The study of these phenomena has been partly completed and its results will be submitted in a later contribution.

Bryn Mawr College

June, 1906

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## DESCRIPTION OF PLATES

The drawings were all made from camera lucida sketches. Zeiss compensating ocular 12, with 2 mm. oil immersion objective, giving a magnification of 1500 diameters, were used for all of the figures except those hereinafter noted. All of the drawings are reduced one-half in reproduction.

(Figs 1-27 from  $CO_2$  eggs)

### PLATE I

- Fig. 1. First polar body formed. Remains of degenerating spindle. Eighteen chromosomes present.  
Fig. 2. Later stage of degenerating spindle. Eighteen chromosomes.  
Fig. 3. Complete disappearance of spindle. Additional chromosomes were in next section of the egg.  
Fig. 4. Chromosomes remaining on a half spindle.  
Fig. 5. Same as Fig. 4, except that the chromosomes have divided.  
Fig. 6. Second polar spindle tangential to surface of egg.  
Fig. 7. Radial position of second polar spindle as seen in later stages.  
Fig. 8. Second polar spindle with eighteen chromosomes.  
Figs. 9 and 10. Early and late anaphase of second polar division.  
Fig. 11. Two polar bodies given off; chromatin lying free in cytoplasm.  
Fig. 12. Formation of female pronucleus through fusion of vesicles.  
Fig. 13. Female pronucleus preparing to divide; chromosomes and fibers appearing.  
Fig. 14. Late prophase of first segmentation division.  
Figs. 15 and 16. Early prophase of first segmentation. Ocular 4, 2 mm. oil immersion objective.



## PLATE II

Fig. 17. Anaphase of first segmentation.

Fig. 18. Vesicle formation preceding formation of daughter nuclei.

Fig. 19. Equatorial plate of Fig. 24.

Fig. 20. Equatorial plate of Fig. 21.

Fig. 21. Section of entire egg through equatorial plate. Ocular 4, 2 mm. oil immersion objective

Fig. 22. Formation of daughter nuclei.

Fig. 23. Metaphase of second segmentation.

Fig. 24. Section of entire egg with chromosomes of equatorial plate divided in first segmentation.

Ocular 4, 2 mm. oil immersion objective.

Fig. 25. Section through equatorial plate of second segmentation spindle.

Fig. 26. Second polar body retained within the egg. Nucleus dividing. Ocular 4, 2 mm. oil immersion objective.

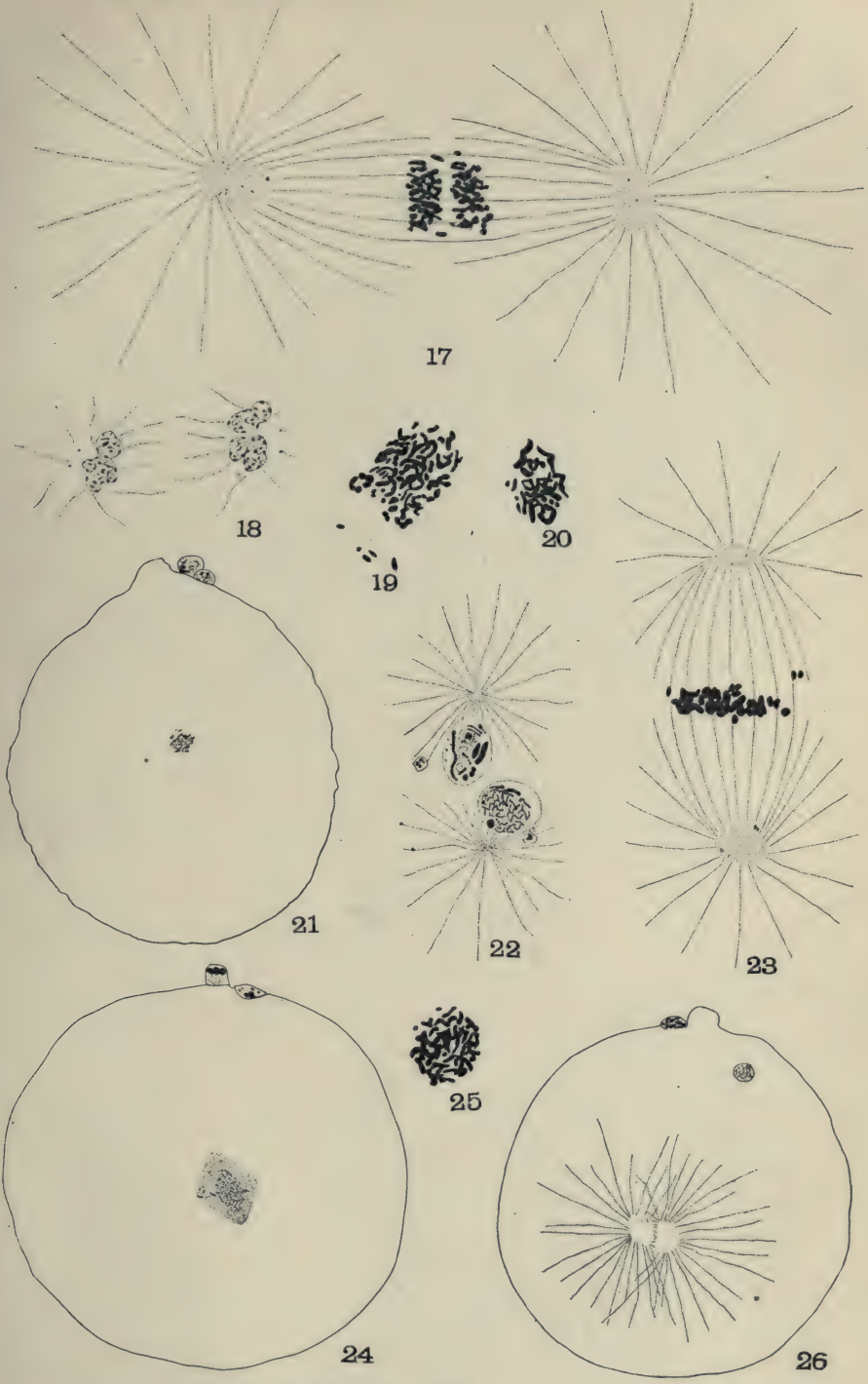


PLATE III

Fig. 27. Second segmentation, slightly earlier than Fig. 23.

Figs. 28 and 29. Adjoining sections of normally fertilized egg in anaphase of first segmentation.

*(Figs 30-69 from CO<sub>2</sub> eggs which were subsequently fertilized)*

Fig. 30. Section through portion of egg immediately after extrusion of first polar body.

Fig. 31. Chromosomes lying free in cytoplasm.

Fig. 32. Tetrad effect due to grouping of bivalent chromosomes.

Fig. 33. Second polar body not extruded. Chromosomes of second polar body lying in cytoplasm beneath the first polar body.

Fig. 34. Second polar spindle. Chromosomes in process of transverse division.

Figs. 35 and 36. Adjoining sections through second polar spindle. Chromosomes in transverse division.

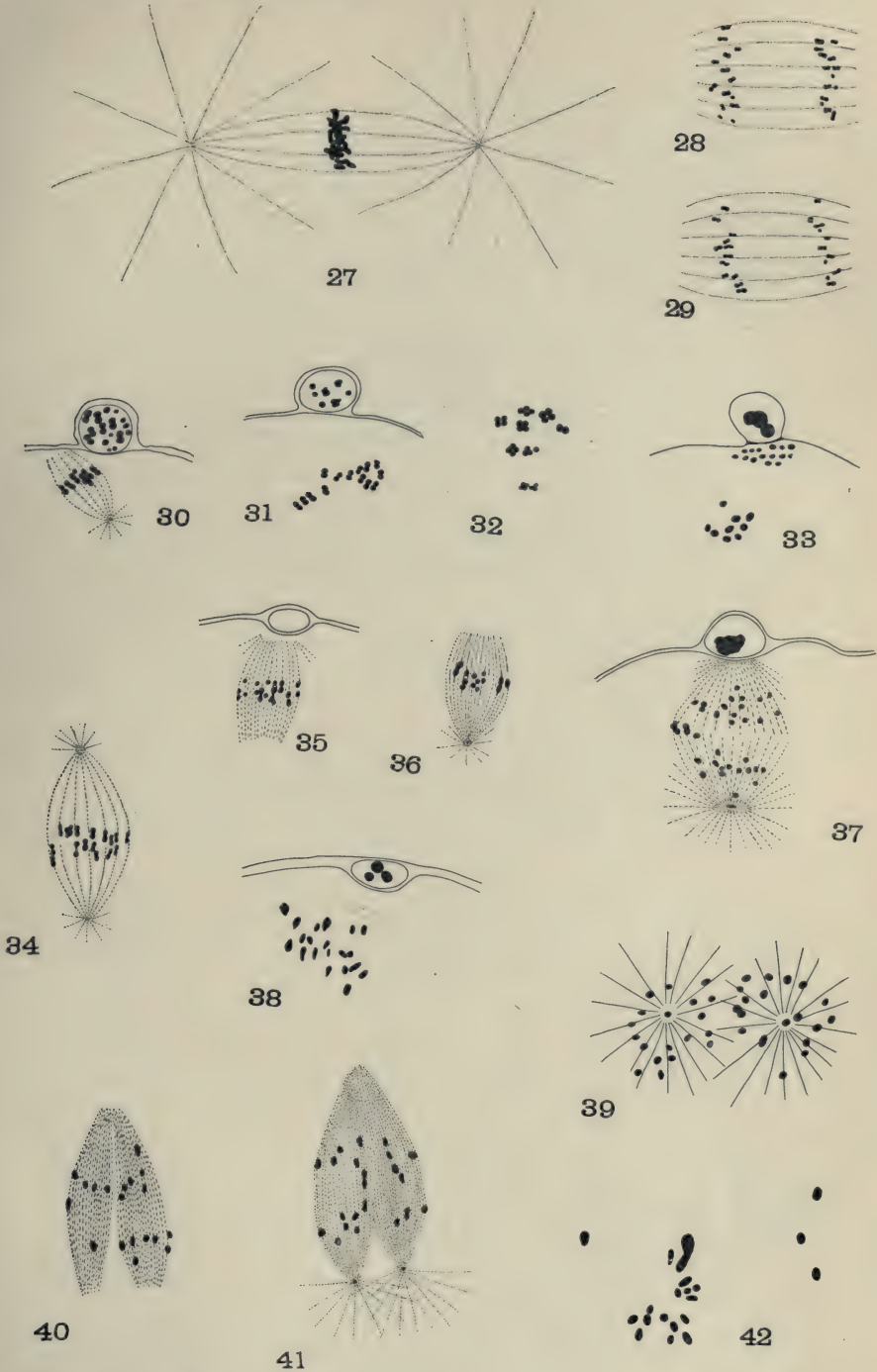
Fig. 37. Second polar spindle. Chromosomes separated into univalent elements.

Fig. 38. Univalent chromosomes remaining in egg.

Fig. 39. Section through long axis of second polar spindle which was lying tangentially to surface of egg.

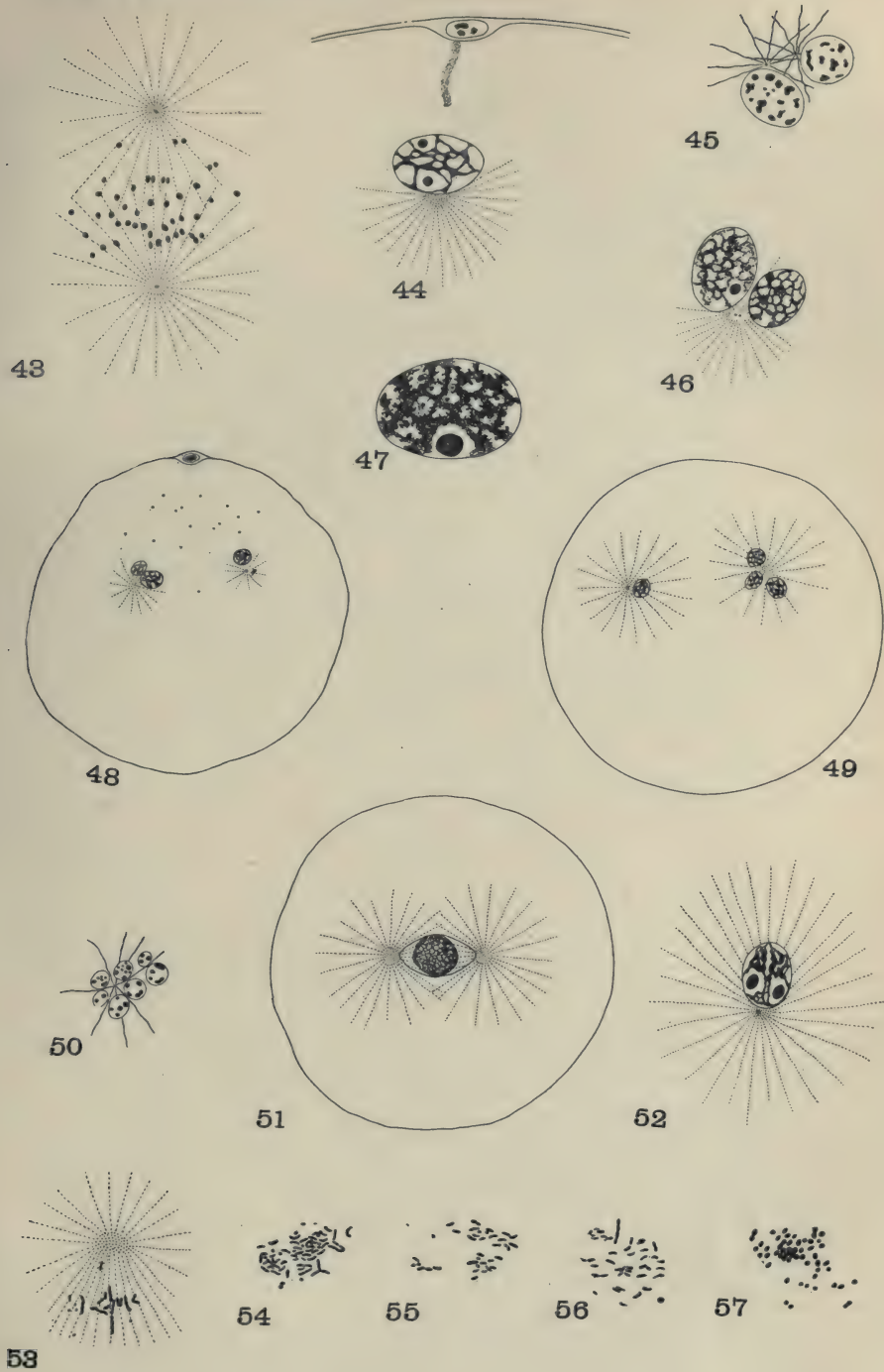
Figs. 40 and 41. Sections through long axis of multipolar spindle.

Fig. 42. Three sections in series through chromosomes remaining in egg after extrusion of second polar body.



#### PLATE IV

- Fig. 43. Second polar spindle formed near center of egg.
- Fig. 44. Female pronucleus formed by fusion of chromosomal vesicles. Part of second polar spindle lying in cytoplasm beneath first polar body.
- Fig. 45. Male and female pronuclei. Aster divided.
- Fig. 46. Male and female pronuclei. Centrosome divided.
- Fig. 47. Segmentation nucleus.
- Fig. 48. Male pronucleus approaching partially formed female pronucleus. Ocular 4, 2 mm. oil immersion objective.
- Fig. 49. Same as Fig. 48.
- Fig. 50. Vesicles which are to fuse and form female pronucleus.
- Fig. 51. Segmentation nucleus elongated; a chromatic fibers are to be seen within the nuclear membrane. Ocular 4, 2 mm. oil immersion objective.
- Fig. 52. Male pronucleus.
- Fig. 53. Portion of first segmentation spindle.
- Figs. 54 and 55. Adjoining sections showing thread broken into short, rod-like segments.
- Fig. 56. Part of section through nuclear material. Three bivalent chromosomes present.
- Fig. 57. Rod-shaped chromosomes rounded up into spherical structures. Some bivalent chromosomes.



## PLATE V

Figs. 58, 59 and 60. Stages in breaking up of segmentation nucleus and of formation of first segmentation spindle.

Fig. 61. Anaphase of first segmentation.

Figs. 62 and 63. Equatorial plates. Univalent chromosomes pairing.

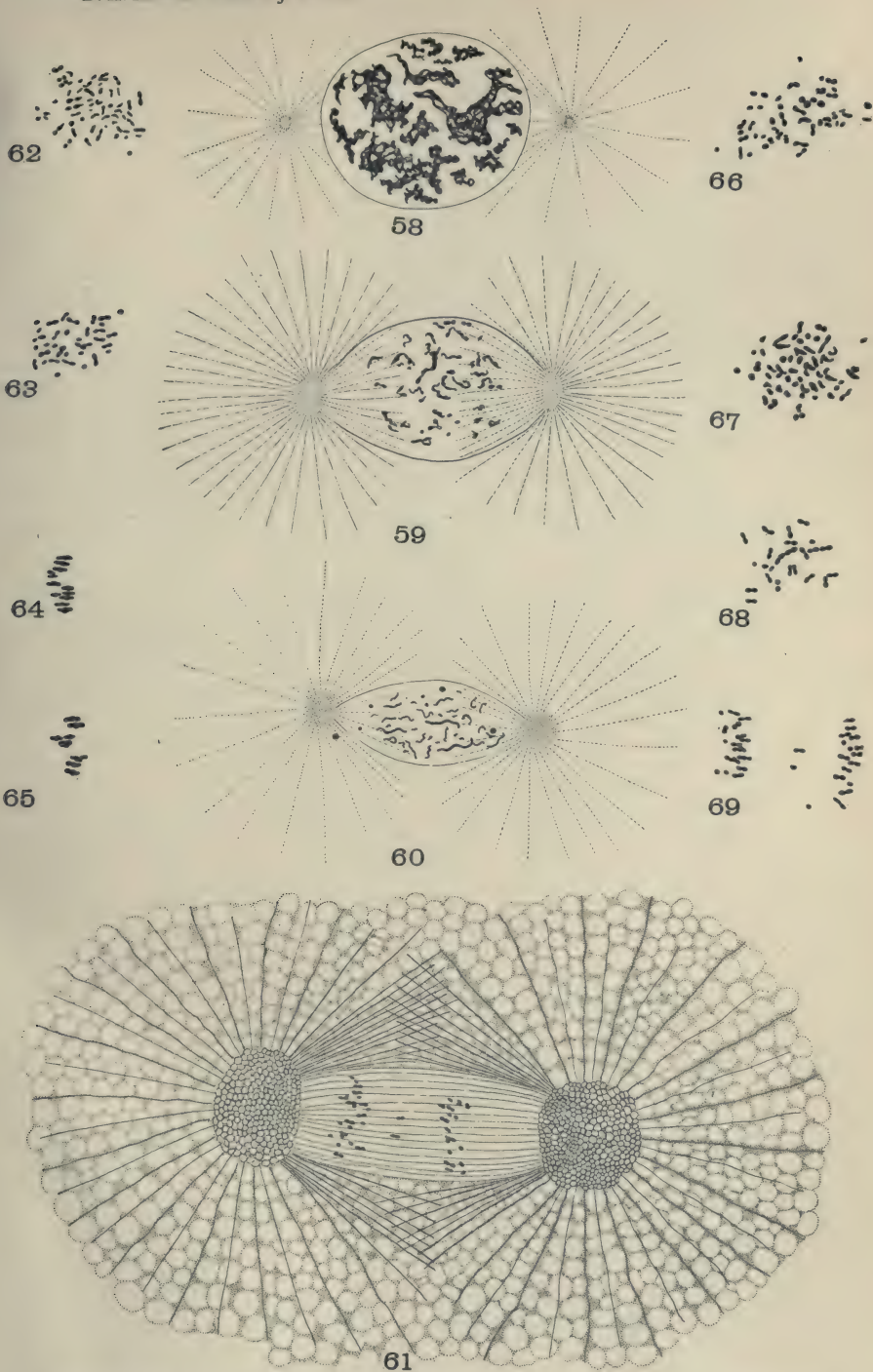
Fig. 64 and 65. Adjoining sections of chromosomes of same spindle showing chromosomes drawn out in first segmentation division.

Fig. 66. Equatorial plate. Chromosomes pairing.

Fig. 67. Same as Fig. 66.

Fig. 68. Equatorial plate just before division, nearly all of the chromosomes being of bivalent form.

Fig. 69. Late anaphase of first segmentation. Same as Fig. 61.





**A Histological Study of Regeneration in *Planaria simplicissima*, *Planaria maculata* and *Planaria morgani*.**

Edited by

**N. M. Stevens.**

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With plates VII—IX and 10 figures in text.

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Eingegangen am 5. April 1907.

The following paper has been written by the editor from notes and drawings made by E. B. O'NEIL, M. J. HOGUE and M. A. CANNON, in the biological laboratories of Bryn Mawr College during the college year 1905—06.

The material used in this comparative histological study consisted of three species of fresh-water planarians: 1) *Planaria simplicissima* Curtis (Text-Fig. B), a dark brown flat-worm which formerly figured in the regeneration papers of MORGAN ('00, '01) and STEVENS ('01) as *Planaria lugubris*; 2) *Planaria maculata* (Text-Fig. A), a large variety with very long auricular appendages, similar in appearance to a California form figured by CHILD in a recent paper ('06 b, Fig. 8, p. 118); and *Planaria morgani* (STEVENS and BORING, '06, Text-Fig. C), a small white planarian, apparently much more rare than the other two.

The object of the work was 1) to compare the method of regeneration of *P. morgani*, — a species which had not been studied from sections, — with that of the better known forms, *P. simplicissima* and *P. maculata*; 2) to settle, if possible several questions of histological differentiation left open by the work of BARDEEN ('01, '02) CURTIS ('02) and STEVENS ('01); 3) to investigate more carefully the comparative rate of regeneration of certain structures at different levels; 4) to compare the regeneration of fission tails in *P. maculata* and *P. morgani*. The last two points had reference to the recent theories

of regeneration advanced by MORGAN ('04a, '04b, '05), and CHILD ('06a, '06b, '06c).

One point which lends especial interest to this comparative study is the fact that *P. morgani* usually multiplies by fission, as does *P. maculata*, while this method of reproduction does not occur in *P. simplicissima*. *P. morgani*, therefore, gives an opportunity for testing further CHILD's hypothesis in regard to the relation between regulation and fission.

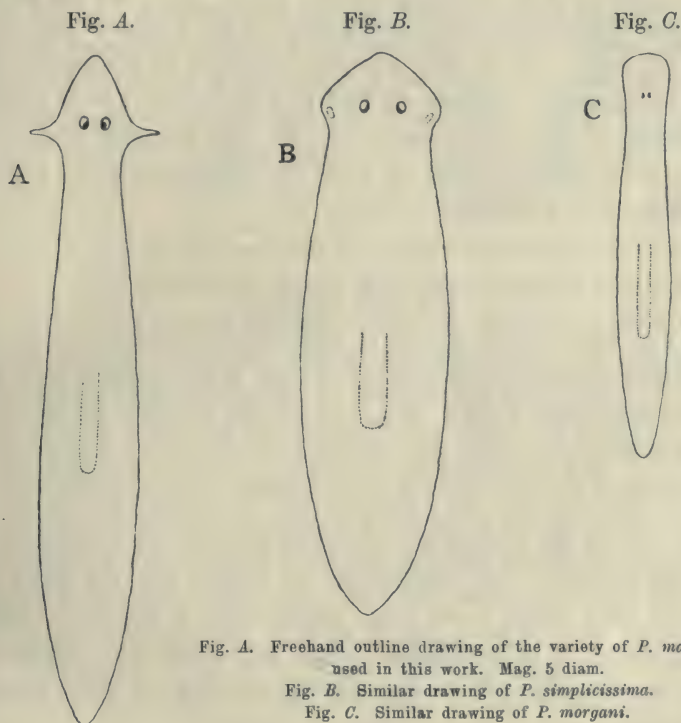


Fig. A. Freehand outline drawing of the variety of *P. maculata* used in this work. Mag. 5 diam.

Fig. B. Similar drawing of *P. simplicissima*.

Fig. C. Similar drawing of *P. morgani*.

### Methods.

For most of the work, the planarians were cut transversely into three nearly equal pieces, which will be referred to as head-pieces, middle-pieces and tail-pieces. These were fixed at various intervals, from a few hours after cutting to ten days, with corrosive acetic (3%) or GILSON's mercurio-nitric mixture. The latter gives more uniformly good fixation. It also causes less curling, and dissolves the rhabdites, which is a decided advantage for the study of the nuclei of the

ectoderm cells. Both sagittal and frontal sections were cut from  $5\mu$  to  $10\mu$  thick, and stained with DELAFIELD's haematoxylin followed by orange G. In studying regeneration of *P. morgani* from living material, the worms were fed with the eye-pigment of the house-fly. After allowing twenty-four hours for the pigment to be ingested, the whole digestive tract was outlined with diagrammatical clearness in dark red against a white background. The animals were then cut and drawings made ten times the size of the pieces as they crawled over millimeter paper pasted under the flat-bottomed glass dish. The color remained distinct in the digestive tract for two weeks or more, and it was therefore possible to distinguish easily in the living worm between old and new endoderm, and to determine the relation of the new pharynx to the old and new parts of the worm. This method is also of value in distinguishing old from new endoderm cells in sections.

### The New Ectoderm.

It has been shown that in *P. simplicissima* (STEVENS, '01) and in *P. maculata* (BARDEEN, '02, THACHER, '02) a cut surface is in a few hours covered over with a thin layer of old ectoderm cells. CURTIS ('02), however, describes the ectoderm of the regenerating end in fission-tails of *P. maculata* as formed from the parenchyma or »formative« cells at the cut surface. He illustrates this point with figures 35 and 36, figures which, judging from our experience with Planarian sections, look suspiciously as though the thin layer of old ectoderm cells might have been accidentally removed in handling after fixation, or in sectioning. This very frequently happens; in fact it is very difficult to get perfect sections of early stages of regeneration.

In *P. simplicissima* cut ends were in some cases completely covered with a very thin layer of ectoderm at the end of six and a half hours (Fig. 1). The ectoderm over the regenerating end remains very thin for about two days, and in the course of two days more becomes nearly as thick as the old ectoderm. Now BARDEEN ('02) states that in *P. maculata* this thickening is the result of an increase in the number of cells by direct division. Many unsuccessful attempts had previously been made to demonstrate either mitosis or amitosis in the ectoderm of *P. simplicissima*. This year fixation with GILSON's mercuronitric fluid, which dissolves the rhabdites, has made the problem easier.

but still no dividing cells have been found in the ectoderm. On examining third and fourth day preparations, however, one frequently finds such appearances as are figured in Figs. 2 and 3; the basement membrane is broken through and cells from the interior are pushing into the ectoderm between two other cells. These migrating cells often contain rhabdites. A similar migration of parenchyma cells into the ectoderm was observed in young embryos of *P. simplicissima* (STEVENS, '04).

In both *P. simplicissima* and *P. maculata* one frequently finds specimens which show, during the first day of regeneration a peculiar heaping up of nuclei in the center of the ectoderm on the cut surface, as though the cells had moved over the injured surface so rapidly that they were for a time piled up in the middle (Fig. 4). A somewhat different heaping up of cells, probably due to contraction in fixation, often occurs on the border of the old part (Fig. 8).

In *P. maculata* the ectoderm over regenerating surfaces is at first thin, as in *P. simplicissima*, but thickens up so rapidly that at the end of the second day it does not differ much in appearance from the old ectoderm (Fig. 5). A special study of the ectoderm at the regenerating surface of fission tails was made by Miss HOGUE and Miss O'NEIL. The results leave no doubt that the closing in is effected in precisely the same way as in pieces severed with a knife; i. e., by old ectoderm cells moving over the exposed tissue. This is shown in Figs. 6, 7 and 8, all from sections of fission-tails recently pinched off, as is indicated by the lack of accumulated parenchyma cells just beneath the layer of ectoderm. The ease with which this thin ectoderm separates from the tissue below is further evidence that it is not formed from parenchyma cells. These and many other similar sections, leave no doubt in our minds that regeneration of the ectoderm in fission-tails proceeds in exactly the same manner as in tails removed with a scalpel, the method of covering the regenerating region being the same as in *P. simplicissima* but more rapid.

In *P. morgani* the wound closes in the same manner. Figure 9 shows a section of a piece 21 hours after cutting. The layer of ectoderm over the cut surface is not so thin as in *P. simplicissima* at this stage, but this Planarian is so extremely thin dorso-ventrally that there is little exposed tissue to cover. By the end of the second day hardly any difference can be detected between the ectoderm over old and new regions. As in *P. maculata*, regeneration of ectoderm in fission-tails does not differ from that in cases of merotomy.

### Ectodermal Gland Cells.

A peculiar new kind of ectoderm cell was discovered by Miss HOGUE in a few 10- and 13-day pieces of *P. simplicissima* (Figs. 10—16). The youngest stage in development observed is shown in Fig. 10, with ordinary ectoderm cells on either side. The cell is here two or three times as broad as the other ectoderm cells and slightly shorter. The nucleus is larger, not so darkly stained, and contains a conspicuous nucleolus. The distal end is ciliated like the other cells. It contains no rhabdites, but distal to the nucleus are elongate masses of a blue staining secretion. The general cytoplasm is homogeneous and apparently denser than that of the other ectoderm cells, resembling somewhat the cytoplasm of young mucous cells in the parenchyma (STEVENS, '01, Taf. XVII Fig. 4). Figure 11 is a slightly later stage where the secretion is beginning to be emitted from the distal end of the cell, where it apparently follows the cilia. The masses around the nucleus are smaller and more numerous, but many of them still have the appearance of streaming out from the nuclear membrane to the distal border of the cell. In Fig. 12 is shown a much larger, and presumably older cell, extending out beyond the general ectoderm. The whole distal surface is covered with a dense brush of long flagella-like threads of secretion which stains blue with haematoxylin, while the ectodermal cilia are unstained. At this stage the globules of secretion have moved entirely away from the nucleus and are massed under the outer cell membrane. All stages between this and one in which the secretion is wholly outside of the cell have been found in the preparations, but in most cases, new masses of secretion begin to appear near the nucleus before the distal end of the cell is quite cleared up. Figure 13 shows the old secretion in long threads with some globules scattered along toward the distal ends and a few small masses of secretion near the nucleus. Figure 14 is a somewhat later stage with a considerable amount of secreted material in the region of the nucleus. This was the largest cell found in these specimens. It will be observed that the form of the cell at this stage is the reverse of that shown in Fig. 10. The basal end is now much narrower than the distal end. On the ventral side of the head such cells as is represented in Fig. 15 were found. Here most of the old secretion seems to have been rubbed off, and a varying number of new masses have been formed near the nucleus in preparation for another secretory period.

These peculiar mucous cells were found in only three specimens, one 10-day and one 12-day head-piece, and one 12-day tail-piece. In the tail-piece they occurred in only one region near the anterior end of the new head. This piece had been fixed in corrosive acetic, and the rhabdites of the other ectoderm cells, as well as poorer fixation made it difficult to follow the different stages of the secretory period. In the other two cases GILSON's mercurio-nitric gave perfect fixation and dissolved the rhabdites, so that the mucous cells stood out with astonishing clearness, their long brushes of blue-staining secretion extending far beyond the border of the general ectoderm. In these pieces the ectodermal gland cells were all in the old part, on both dorsal and ventral sides and at the anterior end. Fig. 16, shows their distribution in a typical sagittal section, about one third of the posterior end being omitted.

So far as previously observed, the mucus secreted by these planarians is formed in mucous cells scattered through the parenchyma, and is thrown out between the ectoderm cells over all parts of the body. This mucus appears to be of three distinct varieties, one staining a deep blue, a second bright yellow, and a third brownish yellow with the DELAFIELD-orange combination.

We could find no evidence of migration of mucous cells into the ectoderm, and this seemed rather unlikely considering the large number of these ectodermal gland cells in certain regions of the two head-pieces. On the other hand, if ordinary ectoderm cells have been redifferentiated into mucus-secreting cells, the rhabdites must either have been ejected or absorbed at an early stage. The relation of the gland cells to the basal membrane and to the neighboring ectoderm cells is the same as that of the ordinary ectoderm cells. There is no evidence that this abnormality is due to parasitism of any kind. The only explanation that we see at present is that ordinary ectoderm cells, disturbed in some way by the changes involved in regeneration, have been redifferentiated into ectodermal, mucus-secreting gland cells. — If this is true, it indicates that the parenchyma cells which were originally differentiated into ectoderm, also contained the potentiality of gland cells, and that this potentiality was not destroyed by differentiation in quite a different direction. An attempt will be made to secure further evidence in regard to the origin of these cells.

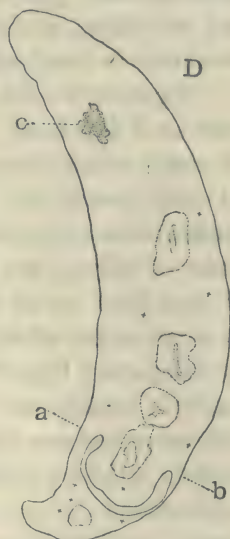
### Migration and Division of Parenchyma Cells.

Sections of pieces fixed 2 days after cutting proved, on the whole, to be the best for a comparative study of the rate and method of accumulation of parenchyma or »formative« (CURTIS) cells in the regenerating region. This gathering of what might also be termed embryonic cells at the cut end is much more rapid in *P. morgani* than in either of the other species. At the end of 48 hours the zone of regeneration is largest both absolutely and comparatively in *P. morgani*, somewhat smaller in *P. simplicissima*, and smallest in *P. maculata*. In *P. maculata* the small accumulation of cells to form a new head or tail-end is associated with the fact that »regulation« is in small measure »regeneration«, in the stricter sense of the word, and in large measure »redifferentiation« (CHILD), while in *P. simplicissima* and especially in *P. morgani* we have extreme cases of »regeneration« with redifferentiation reduced to its lowest terms. It is however, true that the accumulation of cells at the cut surface is more rapid in *P. maculata* than in *P. simplicissima* during the first 24 hours. In all three species mitoses of parenchyma cells were found both in the zone of regeneration and in the old part during the first two days after cutting, less abundant in *P. maculata*, as was to be expected, than in the other species. This reëxamination of this question in *P. maculata* confirms the statements of THACHER ('02) on this point. In all three species abundant evidence of active migration of parenchyma cells from the old to the new part was found. On comparing the portions of the old tissue near the regenerating end with parts far away and with sections of normal animals, it was clear that not only were parenchyma cells dividing more actively in such portions, but they were evidently streaming toward the cut end, turned so as to move in the direction of least resistance, parallel with their longer axes. This is shown in Figs. 1 (*P. sim.* 6½ h.) and 5 (*P. mac.* 2 d.), and might have been better shown in special figures, had it not seemed necessary to avoid too great multiplication of illustrations.

In this connection the question arose whether the rapid lengthening of certain parts in *P. simplicissima* and *P. morgani* in later stages of regulation might be due to increase in the number of cells in the new part by division or to migration of cells from the adjacent old tissue. It is without doubt due in part to the process of differentiation of the rather small and crowded parenchyma cells of the early stages

of regeneration into the larger and more loosely arranged tissue cells of later stages, but this seems not to account fully for the increase in length of the tail region of anterior pieces or of the region between eyes and pharynx in tail pieces of *P. simplicissima* and *P. morgani*. Most active elongation in these two regions was found to be occurring in *P. morgani* from 6 to 8 days, and in *P. simplicissima* 10 days after cutting. Serial sections of 6- and 8-day pieces of *P. morgani* and of 10-day pieces of *P. simplicissima* were carefully examined by Miss HOGUE for mitoses in both old and new parts. A few more cases of mitosis were found in 6- than in 8-day pieces of *P. morgani* (64.5 : 59.5). A typical case for *P. simplicissima* is that of a 10-day head piece in 20 sections of which 66 cells were dividing by mitosis. The division figures were scattered through both the old and the new parts. Text-figure D is an outline drawing from one of the 20 sections showing the location of the dividing cells. The line *a...b* indicates approximately the boundary between old and new parts of the piece. Evidence of migration of parenchyma cells from the old to the new region was also observed. The conclusion was drawn that the regulation in proportion in later stages of regeneration in *P. simplicissima* and *P. morgani* is largely due: 1) to differentiation of the earlier compact mass of embryonic cells into the larger and looser cells of the various tissues; 2) to multiplication and differentiation of parenchyma cells in the new part; and 3) to division of parenchyma cells in the old part and migration of such cells into the new part. There is also, as is evident from study of the living material, more or less gradual slight shifting of tissues en masse, in a longitudinal direction, changing the original piece to a narrower and longer form. This fact comes out clearly in all series of scale drawings made at daily intervals from the time of cutting to 20 days. Attention has been repeatedly called to this point by MORGAN, and sections give less evidence here than repeated observation of the same individual. Such observations are best made on *P. morgani*

Fig. D.



Outline camera drawing of sample section of 10-day head piece of *P. simplicissima* showing location of cells in mitosis. *a...b* indicates the boundary line between old and new parts. *c* brain. ZEISS AA, oc. 2. (H.)

fed with the eye of the house-fly 24 hours before cutting. This point will be illustrated by a series of figures by one of the authors in a future paper.

### Rate of Regeneration of Certain Structures at Different Levels.

This part of the problem was investigated by Miss CANNON, using sections of 5- and 7-day head, middle and tail-pieces of the three species. Eye and brain development were compared in middle and tail-pieces, growth of tail in head and middle-pieces, and pharynx formation in head and tail-pieces.

Figures 17—20 show typical sections of the four regenerating ends in 5-day head, middle and tail-pieces of *P. maculata*. The eye (*e*) and brain (*b*) development in sections 18 and 20 will be seen to be very nearly the same, and this was found to be the rule, with considerable variation in rate of development in different pieces or series of pieces; but development is as often more or less advanced at one level as at the other. The formation of a new pharynx is as a rule more advanced in head than in tail pieces, as may be seen by comparing Fig. 17 with Fig. 20. The pharynx chamber at this stage is usually too large for the pharynx in tail-pieces. The new tail region appears to be longer at this stage in head-pieces (Fig. 17) than in middle pieces (Fig. 19), but in *P. maculata* there is less real regeneration in the tail region and more regulation than in other forms, making comparison difficult. In every case observed in this variety of *P. maculata*, the new pharynx, in pieces cut in front of the pharynx, may be clearly seen in the earliest stages to be forming in the old tissue but so close to the new tail-region that the accumulation of embryonic cells for the pharynx and the tail are continuous at the middle of the section. The new tail therefore begins at the posterior wall of the pharynx chamber. In middle pieces it is more difficult to mark its limits, for the ectoderm quickly thickens up and becomes pigmented over the new part. Figure 21 is an enlarged drawing of brain and eye-spot from the same series of sections as Fig. 20.

Figures 22—25 show a similar set of sections from 5-day pieces of *P. simplicissima*. Again brain and eye development are at about the same stage in middle and tail-pieces (Figs. 23 and 25), the pharynx more advanced in head-pieces (Fig. 22) than in tail-pieces (Fig. 25), and the tail longer in head pieces (Fig. 22) than in middle piece

(Fig. 24), for in *P. simplicissima* the pharynx distinctly belongs to the new part (STEVENS, '01).

In *P. morgani*, though regeneration in general is, as a rule, more rapid than in the other species, the beginning of eye differentiation does not occur until the sixth or seventh day. The seven-day series therefore proved to be the better for comparison of development at different levels. A few figures from two five-day series are given to illustrate other points. The first series was made in October 1906, the second in March 1907. Much to our surprise the second series, through perfectly normal and healthy, was much behind the earlier series, probably due to the fact that the sexual organs were developing rapidly. This point, however is being tested by further experiments. Figures 26—27*b* from the first series show the usual rate of development in five-day pieces, of the pharynx in head-pieces (Fig. 26) and of the brain in middle (Fig. 27*a*) and tail-pieces (Fig. 27*b*). Figures 28—31 are the four regenerating ends from the second series, corresponding to the sets of figures described for the other species. Brain development in middle and tail-pieces was noted as being about equal, though it is shown only in figure 29. The pharynx in head (Fig. 28) and tail-pieces (Fig. 31) showed very closely corresponding stages of development. In most cases the pharynx-chamber was just beginning to open and the axial gut in tail pieces was not yet formed. In the specimen from which Fig. 31 was taken several sections intervened between the two divisions of the old gut, and in these sections new endoderm cells were differentiating (Fig. 31). Another 5-day tail-piece (Fig. 32) shows a slightly more advanced stage, the lumen having appeared in the new part of the gut. Figure 33, a section from a 49-hour piece which contained a part of the old pharynx-chamber illustrates the early gathering of parenchyma cells at the anterior end of the pharynx-chamber before there is any indication either of union of the two lateral divisions of the digestive tract or of differentiation of new endoderm cells. In general it was noted from observations on living pieces of *P. simplicissima* and *P. morgani* that tail-ends of pieces cut anterior to the pharynx develop much more rapidly than those of middle-pieces or of pieces cut posterior to the pharynx. This is in accord with MORGAN's recent studies on the relation between rate of growth and rate of regeneration (MORGAN, '06). Regeneration is most rapid in regions where most growth must occur to replace the missing part. In sections it is impossible to draw a line across a figure and say that on one side

you have the new part and on the other the old, for the new pharynx, though in early stages plainly connected with the regenerating head or tail-end, is, as it were, inserted into the border of the old part.

Figures 34—40 are from a 7-day series. Sections 34 and 35 represent a prepharynx-piece (Fig. 34) and a tail-piece. Each contains a 1-cell eye-spot, and brain development is nearly the same in the two pieces. Figures 36—38 are higher-power drawings of 1- and 2-cell eye-spots, 36 from a tail-piece, 37 from a middle-piece and 38 from a prepharynx-piece. The average rate of development of these two structures is about the same at widely different levels, although head development as a whole is more rapid in anterior pieces. As to rate of pharynx development at different levels in *P. morgani*, several series of daily scale drawings show that in general the prepharynx-pieces are in advance of postpharynx-pieces in this respect. A fairly typical 7-day pharynx regeneration is depicted in Figs. 39 (head-piece) and 40 (tail-piece). The apparent separation between the pharynx and the head in Fig. 40 is due to rapid differentiation and growth in the region between brain and pharynx. In early stages (Figs. 31 and 32) the new pharynx and the regenerating end are always closely connected. This is well shown in a sagittal section of a young fission-tail (Fig. 41) where the split in the parenchyma cells for the pharynx chamber has just appeared. The massed parenchyma for pharynx and head are continuous on the ventral side while the old digestive tract appears at a point farther anterior on the dorsal side (section cut slightly oblique). Figure 42 is from an older fission-tail, where the two lateral divisions of the gut have united in front of the new pharynx and made connection with it. This together with rapid differentiation has separated the pharynx from the head region. The pharynx may usually be clearly seen in the living piece on the fourth day and often on the third day, while in *P. simplicissima* and *P. maculata* it can rarely be found in sections before the fifth day. *P. morgani* is therefore in advance of the other species in time of pharynx development, but behind both in eye and brain development. The amount of new tissue compared with the old is always greater in *P. morgani* than in either of the others, for pieces cut at the same time.

It will readily be seen that our results give very little support to MORGAN'S theory of gradation of organ-building materials in the planarian body. In *P. morgani* and *P. simplicissima* rate of deve-

lopment of the pharynx in head and tail-pieces should be the same, as the pieces were cut, on the average, at equal distances in front of and behind the pharynx chamber. The pharynx, however, seems usually to be somewhat better developed in head than in tail-pieces of the same age. Again the brain and eyes in anterior pieces minus the old head and in middle pieces should be much in advance of the same structures in tail pieces, but this is not the case (Figs. 18, 20, 23, 25, 34, 35). If there were such a gradation of materials as MORGAN supposes, it would seem that eyes and brain should develop much more slowly or less perfectly in posterior than in anterior pieces.

Little dependence can be put on comparative measurements of head and tail regeneration at different levels, either in living pieces or in sections, on account of differences in longitudinal contraction and the central position of the normal pharynx makes it not so good a test of the theory as an organ situated near one end of the animal. The rate of development of brain and eyes seems on the whole to be the best test of the possible influence of a gradation of organ-forming materials on the rate of regeneration at different levels in these Planarians.

### Fission-Tails.

We have been especially interested in the fission-tails of *P. morgani* in connection with the difference in the position of the new pharynx as compared with *P. maculata*. In *P. morgani* the pharynx in fission-tails always appears just within the border of the old tissue but, as shown in Fig. 41, in direct connection with the accumulated parenchyma cells at the head end. The pharynx can be seen with perfect clearness in the living pieces under a hand lens, or better under the low power of a compound microscope, often on the third day, and always on the fourth, as a clear shining spot between the two lateral divisions of the digestive tract. Now fission cuts off the tail-piece in *P. morgani* either immediately behind the pharynx or at a variable short distance farther back, exactly as in *P. maculata*. Epidemics of fission of both species have occurred in the laboratory aquaria, and many specimens of both kinds have been carefully watched, fixed and sectioned at various stages. In the tail-pieces of this long-eared variety of *P. maculata*, the pharynx appears in the old tissue from  $\frac{1}{4}$  to  $\frac{1}{3}$  of the distance from the anterior to the posterior end of the piece. The distance is nearer  $\frac{1}{4}$  in the very longest pieces

whether obtained by normal fission or by merotomy, and about  $\frac{1}{3}$  in all other tail pieces whether cut off near the pharynx or much farther toward the end of the tail.

We have found it very difficult to understand how preparation for fission could account for such a variation in the location of the pharynx as CHILD figures on page 115, Fig. 5 *c* and *d* and page 116, Fig. 7 *b* and *e* of his recent paper on »The Relation between Regulation and Fission in *Planaria*«. In *P. morgani* we have a Planarian in which the relation of the new pharynx to old and new parts of the regenerating tail-piece is uniform whether the piece be obtained by fission or by cutting at any level behind the pharynx. In this case regulation consists almost entirely of regeneration rather than of redifferentiation, a long prepharyngeal region being developed with little change in the old part. According to CHILD's theory of regulation, the functional center of all tail-pieces of *P. morgani* is near the anterior end, while that of corresponding pieces of our variety of *P. maculata* is about  $\frac{1}{3}$  of the length from the anterior end. The uniformity of conditions in all regenerated tail-pieces of these two forms, both of which divide by fission, makes us doubt whether preparation for fission had anything to do with the irregularity discussed by CHILD in the paper referred to above.

#### Development of a new Pharynx in the old Pharynx-Chamber.

A few preliminary experiments with *P. morgani* having shown that if the pharynx is removed by a cut posterior to the base of the pharynx so as to leave a part of the pharynx-chamber in the piece, the chamber does not disappear, but the new pharynx comes in at its anterior end. The development of the pharynx in such pieces was studied by Miss HOGUE in *P. morgani* and *P. simplicissima*. Figures 43—46 give a series of stages from 36 hours to 4 days for *P. morgani*, and Figs. 47—49 the earlier stages in *P. simplicissima*, from 18 to 36 hours. In both cases it was plain that parenchyma cells accumulate first at the cut surface, but some of them soon begin to migrate to the anterior end of the pharynx-chamber, which shrinks back so as to fall somewhat short of reaching the cut end. The new pharynx consequently forms just within the old tissue but connected with the new head region so far as the origin of its cells is concerned. The early migration was often one sided, as seen in Figs. 44 and 49, but all older pharynges were symmetrically formed. The rate of development of the pharynx in the old chamber was

more rapid and the time of its appearance earlier than in pharynx regeneration in any other region. This may easily be supposed to be due to some stimulus resulting from the presence of the empty chamber. A study of the whole series of sections from 36- and 48-hour pieces shows that at this time there is no axial gut. We have therefore plenty of evidence that pharynx formation is not dependent on the presence of an axial gut (BARDEEN, '01), the regeneration of pharynx and gut being simultaneous phenomena as suggested by LILLIE ('01).

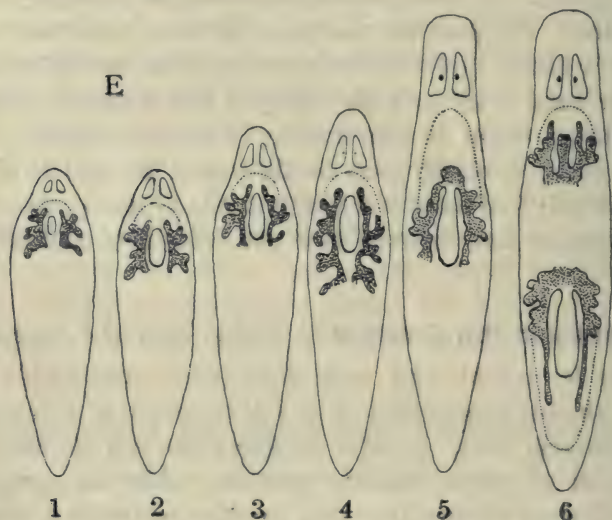
In connection with the experiment just described, a slightly different one was tried. The pharynx was removed from whole worms with a straw or a needle, and then the prepharynx part cut off at different levels, or in some cases only the pharynx was removed. The new pharynx always comes in very rapidly in the old chamber. In *P. morgani* it could be clearly seen on the second day and by the sixth day had reached  $\frac{2}{3}$  of its normal length, a rate of regeneration at least twice as rapid as that observed in 5- and 7-day head and tail-pieces.

#### Development of New Endoderm in Fission-Tails of *P. morgani*.

Figures 50 and 51 were made from frontal sections of a fission-tail which showed especially well the development of new endoderm from parenchyma cells. In this specimen the old endoderm was unusually full of yellow staining granules, while the cytoplasm of the new endoderm (*e*) was free from granules and stained blue. The contrast is fairly well shown in the figures. It is also evident that the new endoderm grades into the parenchyma, division walls being difficult to distinguish in many cases. These figures show clearly that in *P. morgani* the old branches of the gut do not unite, but the space between is filled in with new endoderm formed from parenchyma tissue. The origin of new endoderm had been left somewhat in doubt by CURTIS ('02) and by STEVENS ('01). Figs. 52 and 53 are portions of new and old endoderm much more highly magnified.

In the living tail pieces of *P. morgani*, previously fed with eye pigment of a fly, the new endoderm is opaque white in contrast with the dark red regions of the old digestive tract and with the translucent white parenchyma. This form therefore gives an excellent opportunity for studying the relation between new and old tissues in the living animal at successive stages in the process of regeneration and regulation.

This point was investigated by Miss O'NEIL in connection with the study of sections of fission-tails referred to above. Drawings were made on millimeter paper, magnified 10 times, and Text-Figs. *E* 1, 2, 3, 4, 5, 6 have been traced from the original drawings. The 5-, 6-, 7- and 8-day drawings (1, 2, 3, 4) show the old digestive tract (stippled) not united in front of the pharynx, but the space filled in with new endoderm (within the dotted line). The old parts of the digestive tract, however, slowly push forward and in the 11th day

Fig. *E*.

1, 2, 3, 4, 5. A series of scale drawings from a tail piece of *P. morgani* fed with eye-pigment of a fly, to show the forward movement of the old gut and its ultimate union in front of the new pharynx. The drawings were made on millimeter paper, mag. 10 diam., on the 5th, 6th, 7th, 8th, and 11th day. The dotted lines show the extent of new endoderm. No. 6 is an 11-day prepharynx piece from the same worm to show both forward movement of the old digestive tract in the head region, and backward movement in the tail region. (N.)<sup>1</sup>

drawing (5) they come together in front of the pharynx. A similar backward growth of the old parts of the digestive tract is even more conspicuous in a series of drawings made from a piece cut out in front of the pharynx. The 11-day drawing is reproduced in Fig. *E* 6. In all these drawings the dotted line shows the extent of the new endoderm.

This movement of the old gut is interesting as a visible demonstration of the shifting of formed tissue during the process of regulation in a Planarian in which there is much less change in form of the old part than in either *P. maculata* and *P. simplicissima*.

<sup>1</sup> N. = O'NEIL; H. = HOGUE; C. = CANNON.

### Abnormal Structures in *P. morgani*.

Miss O'NEIL reports the following abnormalities observed in *P. morgani*: An interesting case of abnormal eye-spots occurred in four specimens of this Planarian in the course of regeneration. One worm was cut into eight pieces. On the seventh day, it was noted that the head-piece showed small pigment spots in the vicinity of the normal eyes. The piece next behind the head had two small eye-spots in normal position. On the eleventh day the second piece showed supernumerary eye-spots irregularly distributed, and the head-piece was dotted with small pigment spots. Later both pieces were fixed and sectioned. Figure 54 is from a section of the second piece, showing a normal eye and a large ball of eye-pigment in the old endoderm (*p*) Figure 55 is a drawing of the anterior part of the section with higher magnification, showing the granular pigment-ball in the endoderm. Figure 56 was drawn from an entire section of the head-piece showing scattered pigment spots (*p*) both in the endoderm and in the parenchyma, and Fig. 57 is an enlarged drawing of the anterior portion of the old endoderm of an adjacent section, showing several masses of pigment (*p*) in the endoderm cells. Figure 58 is a densely pigmented cell taken from the parenchyma of section 56. Three other similar cases of supernumerary eye-spots were noted, two short head-pieces and one long prepharynx piece. The appearance of extra eye-spots in the parenchyma was not so surprising, for occasional cases of supernumerary eyes had previously been noted in regenerated heads, but the development of masses of pigment exactly like that in the eyes, in old endoderm cells, was most unexpected. Nothing of the kind has ever been found in normal worms, or in the hundreds of pieces studied alive and in sections. As these pigment spots were observed developing at the same time as the normal eyes, the stimulus which caused them to appear would seem to be in some way connected with the process of regeneration, the equilibrium of all the tissues probably being somewhat disturbed. We were unable to detect anything peculiar in the environment which might account for this abnormality, which in some respects resembles the development of mucus-secreting cells in the ectoderm, described in an earlier part of the paper. Considered in relation to the fact that in these Planarians, any normal structure may be regenerated at any level between the brain and the tip of the tail, these phenomena give some support to the idea that all

of the so-called parenchyma, formative, or embryonic cells may contain the potentiality of development into any one of the various kinds of differentiated organ or tissue cells. Whether this pigment-forming potentiality belongs to cytoplasm or nucleus is not evident from the sections. The pigment masses are found in various parts of the endoderm cell, and occasionally more than one cell is involved, but nothing even suggesting a complete eye occurs.

One case of two pharynxes in one pharynx-chamber was found in sections of a 6-day piece cut just behind the pharynx, and including half of the tail. Text-figure *F* shows a longitudinal section of one

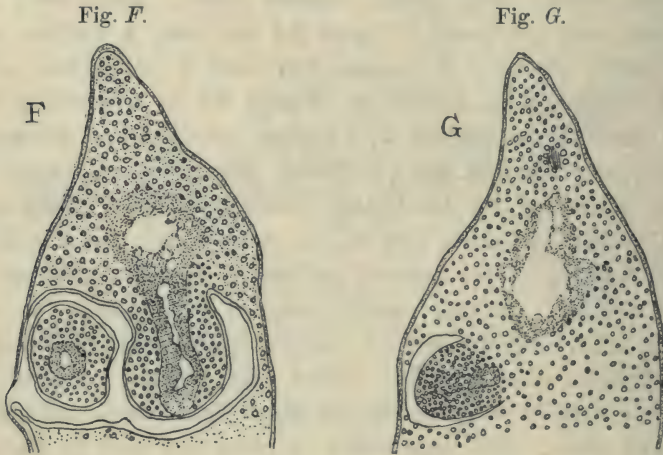


Fig. *F* and *G*. Sagittal sections showing two pharynxes in one pharynx-chamber. Six-day piece of *P. morgani*. ZEISS AA, oc. 8. (N.)

pharynx, and a cross section of the other, and Fig. *G* shows the attachment of the second pharynx; both were connected with the axial gut.

In another lot where many died, probably from something unfavorable in the conditions, two abnormal forms resulted. One had two tails (Text-Fig. *H*) and another (Text-Fig. *L*) folded together at the anterior end forming a ventral pocket, as often happens in *Dendrocoelum lacteum* and *Polychoerus caudatus*, and no head developed. Both of these cases came from short cross pieces cut either through the pharynx region, or just posterior to it.

A peculiar double-headed form developed from a piece, the anterior end of which came from the pharynx region of the original worm (Text-Fig. *M*). One of the heads presents a normal appearance, but the other has the sides folded under and united forming a pocket, suggesting that it may be a case of successful head development

after such a closing of the anterior end as appears in Fig. *L*. The branching of the endoderm is very irregular and sections showed only two complete longitudinal nerve cords, one in each piece, while another cord connected the two heads. The drawing (Fig. *M*) was made when the piece had been regenerating about three weeks. It was thought that the development of two heads might be due to the independent influence of the two nerve cords separated by the empty pharynx-chamber, but all attempts to secure more double-headed or double-tailed animals from pieces cut in the pharynx region failed.

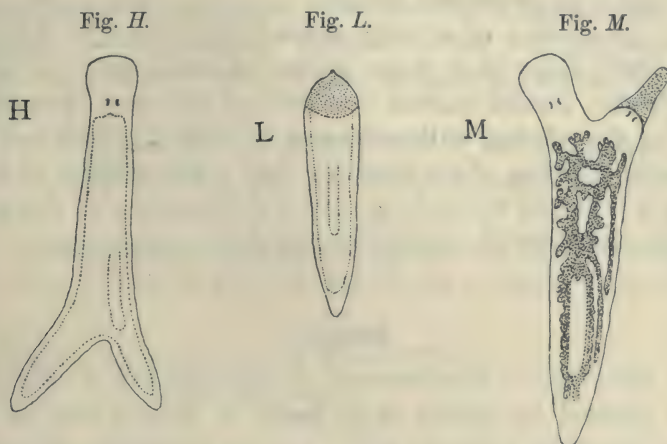


Fig. *H*. Scale drawing of double-tailed piece of *P. morgani* from the pharynx region.  
Mag. 10 diam. (N.)

Fig. *L*. Scale drawing of regenerated piece of *P. morgani*, with a ventral pocket in place of a head.  
Mag. 10 diam. (N.)

Fig. *M*. Scale drawing of a double-headed piece of *P. morgani*. Mag. 10 diam. (N.)

In our collections one worm was found having a second head, pre-pharynx region and a pharynx extending into the normal pharynx-chamber. This we supposed must have resulted from a bite or other injury to the side of the animal, but we have been unable to produce such forms in the laboratory.

In Fig. 59 Miss O'NEIL has shown several groups of cells (*y*) which are evidently the same as those marked *x* in CURTIS's Fig. 41. These were found in many fission-tails of *P. maculata*, but not in the other species. CURTIS reports that these structures were found in most of the specimens examined from localities where fission was occurring. He says, »There are lying in the parenchyma, mostly in the dorsal region, masses of large cells, outlines of which are not always easily discernible. They are not connected with the gut in any way, nor do they resemble

any structure which I have ever found described for the *Planaria*. They can best be characterized by saying that they have the appearance of gut cells without any vacuoles, for their nuclei are very much like those of the gut and their cytoplasm stains in the same way, but they are not found connected with the gut in any way. They are furthermore not at all like the first beginnings of the reproductive organs and although I have held many theories as to their significance I cannot offer even a plausible conjecture as to their meaning. My reason for mentioning them is that they are usually a striking feature of animals from an asexual locality.

An examination of a large number of sections of *P. maculata* leaves little doubt that these groups of cells must be dormant yolk glands. Lateral sagittal sections are the most favorable for observing the relation of these groups of cells (Figs. 59 and 60) to the lateral branches of the digestive tract. The relation of the two structures is exactly the same as that of the branches of the gut and the yolk glands in *P. simplicissima* in the breeding season.

### Summary.

1) On *Planaria simplicissima*, *P. maculata* and *P. morgani*, a surface exposed by cutting or by fission is covered over by a thin layer of migrating ectoderm cells. Later, parenchyma cells migrate into the ectoderm of the new part (Figs. 2 and 3).

2) A new kind of ectodermal gland cell is described for *P. simplicissima* (Figs. 10—15).

3) Accumulation of parenchyma cells at a cut end of regenerating pieces is in large measure due to migration from the old part in the three species.

4) Migration is also an important factor in the later process of growth and regulation in *P. simplicissima* and *P. morgani*.

5) Brain and eyes regenerate at about the same rate in prepharynx and postpharynx pieces.

6) The pharynx regenerates somewhat more rapidly in prepharynx than in postpharynx pieces, and most rapidly in the empty pharynx chamber or a part of it.

7) In *P. simplicissima* the pharynx always regenerates in the new part but on the border of the old. In this variety of *P. maculata* the pharynx at levels anterior to the pharynx appears in the old tissue, but on the border of the new, and at levels behind the pharynx

from  $\frac{1}{4}$ — $\frac{1}{3}$  of the length of the piece from the anterior end. In *P. morgani* the pharynx at all levels develops in the old tissue but on the border of the new, the accumulation of parenchyma cells being continuous in early stages.

8) Preparation for fission has no influence on the method of regeneration of tail-pieces in *P. morgani*.

9) Regulation in *P. morgani* consists almost entirely of regeneration with very little redifferentiation.

10) In tail pieces of *P. morgani* the axial gut is formed from newly differentiated endoderm cells after the pharynx has begun to develop, but the lateral divisions of the old gut may later (11 days) move forward so as to unite in front of the pharynx (Text-Fig. E 1—5).

11) Development of eye-pigment in old endoderm cells gives some support to the idea that all embryonic cells in *Planaria* are totipotent.

12) The groups of cells figured by CURTIS ('02) as *x* in Fig. 41 and by Miss O'NEIL in Figs. 59 and 60 are probably dormant yolk glands.

Biological Laboratory, Bryn Mawr College,  
March 23, 1907.

### Zusammenfassung.

1) Bei *Planaria simplicissima*, *Pl. maculata* und *Pl. morgani* wird eine durch einen Schnitt oder durch Spaltung exponierte Wundfläche mit einem dünnen Lager von wandernden Ectodermzellen überzogen. Später wandern Parenchymzellen in das Ectoderm des neuen Teils (Fig. 2 und 3).

2) Eine neue Art ectodermaler Drüsenzelle wird für *Pl. simplicissima* beschrieben (Fig. 10—15).

3) Die Anhäufung von Parenchymzellen an einem abgeschnittenen Ende von regenerierenden Stücken ist in großer Ausdehnung bewirkt durch Einwanderung vom alten Teil her, und zwar bei allen drei Arten.

4) Die Zellwanderung ist auch bei dem späteren Wachstums- und Regulationsprozeß ein wichtiger Faktor bei *Pl. simplicissima* und *Pl. morgani*.

5) Gehirn und Augen regenerieren sich annähernd in demselben Betrage bei vor dem Pharynx wie bei hinter ihm belegenen Stücken.

6) Der Pharynx regeneriert etwas schneller in präpharyngealen als in postpharyngealen Stücken und am schnellsten in der leeren Pharynxkammer oder einem Teile derselben.

7) Bei *Pl. simplicissima* regeneriert der Pharynx immer in dem neuen Teil, aber am Rande des alten. Bei der Varietät *Pl. maculata* erscheint der Pharynx, falls das Stückniveau vor dem Pharynx liegt, im alten Gewebe, aber am Rande des neuen, und bei hinter dem Pharynx belegenen Niveau um  $\frac{1}{4}$  bis  $\frac{1}{3}$  der

Stücklänge vom Vorderende entfernt. Bei *Pl. morgani* entwickelt sich der Pharynx für jedes Niveau innerhalb des alten Gewebes, jedoch am Rande des neuen, indem die Anhäufung von Parenchymzellen auf frühen Stadien kontinuierlich stattfindet.

8) Die Vorbereitung zur Spaltung hat keinen Einfluß auf die Regenerationsmethode von Schwanzstücken von *Pl. morgani*.

9) Die Regulation besteht bei *Pl. morgani* fast gänzlich aus Regeneration mit nur sehr wenig Wiederdifferenzierung.

10) Bei Schwanzstücken von *Pl. morgani* wird der axiale Darmteil aus neuerdings differenzierten Entodermzellen gebildet, nachdem der Pharynx sich zu entwickeln begonnen hat, aber die seitlichen Verzweigungen des alten Darmes können später (11 Tage) nach vorn rücken, so daß sie nach vorn vom Pharynx miteinander sich vereinigen (Textfig. E 1—5).

11) Die Entwicklung von Augenpigment in alten Entodermzellen verleiht dem Gedanken eine gewisse Stütze, daß bei *Planaria* alle embryonalen Zellen totipotent sind.

12) Die von CURTIS (1902)  $\alpha$  in Fig. 41 und von Frl. O'NEIL in Fig. 59 und 60 abgebildeten Zellgruppen sind wahrscheinlich ruhende Dotterdrüsen.

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## Description of Plates.

### Plate VII.

- Fig. 1. Cut end of *P. sim.* 6½ hours, showing thin layer of ectoderm over the cut surface. LEITZ obj. 3, oc. 12. (C.)<sup>1)</sup>
- Figs. 2—3. Parenchyma cells (*m*) migrating into the ectoderm. 3-day regenerating piece of *P. sim.* ZEISS 2 mm., oc. 8.
- Fig. 4. 24-hour piece of *P. sim.* showing heaping up of ectoderm (*n*) at the center of the cut surface. ZEISS D, oc. 6. (H.)
- Fig. 5. 2-day regenerating end of *P. maculata*, sag. section. LEITZ obj. 3, oc. 12. (C.)
- Fig. 6. Recently closed in end of a fission-tail of *P. mac.* showing thin migrated ectoderm (*e*). ZEISS D, oc. 6. (H.)
- Fig. 7. Regenerating end of fission-tail of *P. mac.*, sag. section. ZEISS AA, oc. 12. (N.)
- Fig. 8. Frontal section of recently closed in fission-tail of *P. mac.*, ectoderm very thin and easily separable from the underlying parenchyma. LEITZ obj. 3, oc. 2. (N.)
- Fig. 9. 21-hour piece of *P. morg.*, closed in, but no accumulation of embryonic cells at the end. ZEISS D, oc. 6. (C.)
- Figs. 10—15. Ectodermal mucous cells from a 10-day head piece of *P. sim.* ZEISS 2 mm., oc. 6. (H.)
- Fig. 16. Outline drawing of anterior 2/3 of a section showing location of mucous cells (*g*). ZEISS AA, oc. 2. (H.)
- Figs. 17—20. Typical sag. sections of *P. mac.* showing development of brain (*b*), eyes (*e*) and pharynx (*p*) at different levels in 5-day pieces. LEITZ obj. 3, oc. 2. (C.)

### Plate VIII.

- Fig. 21. 5-day regeneration of brain (*b*) and eye (*e*) from the same series of sections as Fig. 20. LEITZ 1/12, oc. 2. (C.)

<sup>1)</sup> N. = O'NEIL; H. = HOGUE; C. = CANNON.

- Figs. 22—25. Typical series from 5-day *P. sim.* LEITZ obj. 3, oc. 4. (C.)
- Fig. 26. Posterior end of 5-day head piece of *P. morgani*. LEITZ obj. 3, oc. 4. (C.)
- Figs. 27a and 27b. Anterior ends of middle-piece (27a) and tail-piece (27b) of *P. morg.*, 5-days, showing brain development (b). LEITZ obj. 3, oc. 4. (C.)
- Figs. 28—31. Typical set of regenerating ends from a somewhat delayed 5-day series showing brain (b) and pharynx (p). LEITZ obj. 3, oc. 4. (C.)
- Fig. 32. A section from a slightly more advanced tail-piece showing lumen in the new endoderm (g) while Fig. 31 shows a solid mass of new endoderm (e). LEITZ obj. 3, oc. 4. (C.)
- Fig. 33. Anterior end, sag. section, of a 49-hour piece showing migration of embryonic cells to the anterior end of the old pharynx-chamber, to form the new pharynx. LEITZ obj. 3, oc. 4.
- Fig. 34. Sag. section from a 7-day head piece of *P. morg.* with the head removed just behind the brain, showing new brain (b) and one pigmented eye-cell (e). LEITZ obj. 3, oc. 4. (C.)
- Fig. 35. Similar section from a 7-day tail-piece showing about the same stage of eye (e) and brain (b) development. LEITZ obj. 3, oc. 4. (C.)
- Fig. 36. Enlarged drawing of the single eye-cell in sec. 35e. LEITZ  $\frac{1}{12}$ , oc. 4. (C.)
- Fig. 37. 2-celled eye-spot from a 7-day middle piece of *P. morg.* LEITZ  $\frac{1}{12}$ , oc. 4. (C.)
- Fig. 38. 2-celled eye-spot from a 7-day piece cut out between the head and pharynx. LEITZ  $\frac{1}{12}$ , oc. 4. (C.)
- Fig. 39. 7-day head-piece showing new pharynx (p). LEITZ obj. 3, oc. 4. (C.)
- Fig. 40. 7-day tail-piece showing brain (b) and pharynx. LEITZ obj. 3, oc. 4. (C.)
- Fig. 41. Sag. section of fission-tail of *P. morgani*, showing new pharynx coming in on the ventral side in connection with the accumulated parenchyma at the head end. ZEISS AA, oc. 8. (H.)
- Fig. 42. Older fission-tail of *P. morg.*, pharynx and head-end separated by differentiation of the new tissue. ZEISS AA, oc. 8. (H.)
- Figs. 43—45. Three stages in the regeneration of a new pharynx in a part of a new pharynx in a part of the old pharynx-chamber in *P. morgani*. Frontal sections. Figs. 43—44, 36 hours; Fig. 45, 2 days. ZEISS AA, oc. 8. (H.)

#### Plate IX.

- Fig. 46. A later stage (4 d.) in the same series as Figs. 43—45. ZEISS AA, oc. 8. (H.)
- Figs. 47—49. Three stages in the formation of a new pharynx in the old pharynx-chamber of *P. sim.* Fig. 47, 18 hours; Figs. 48—49, 36 hours. ZEISS AA, oc. 8. (H.)
- Figs. 50—51. Frontal sections of a fission-tail of *P. morg.* showing new ectoderm (e) between the lateral divisions of the old gut. ZEISS AA, oc. 8. (N.)
- Figs. 52—53. Small portions of new and old endoderm respectively, more highly magnified. ZEISS D, oc. 8. (N.)
- Fig. 54. Sag. section of prepharynx piece of *P. morgani*, showing pigment-spots in the endoderm (p). BAUSCH & LOMB obj. 1, oc. C. (N.)
- Fig. 55. Anterior portion of same, more highly magnified. BAUSCH & LOMB obj.  $\frac{1}{5}$ , oc. C. (N.)

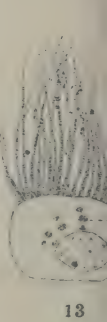
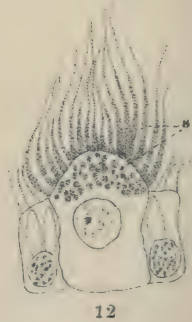
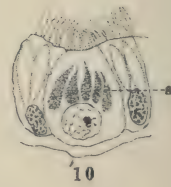
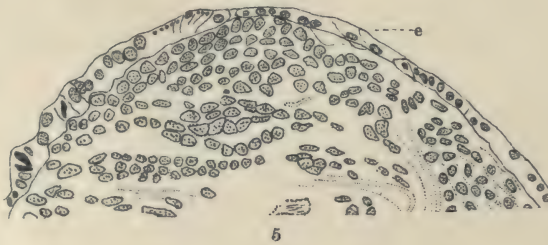
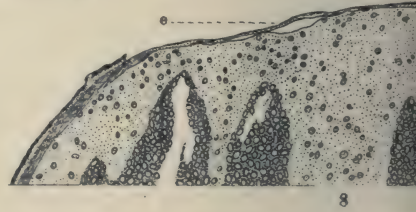
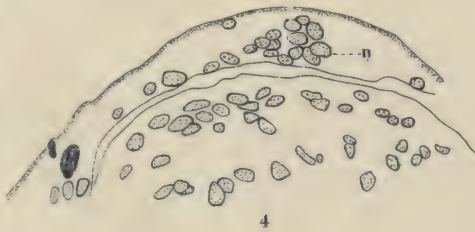
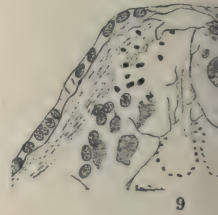
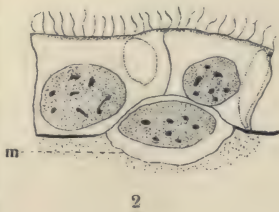
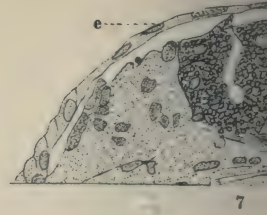
- Fig. 56. Sag. section of head-piece from same individual as Figs. 54—55, showing many scattered pigment (*p*) spots posterior to the brain region (*b*). BAUSCH & LOMB obj. 1, oc. C. (N.)
- Fig. 57. Portion of digestive tract from an adjacent section of the same piece, showing masses of eye-pigment in the old endoderm cells. BAUSCH & LOMB  $\frac{1}{12}$ , oc. A. (N.)
- Fig. 58. Cell from the parenchyma of section 56, showing the cytoplasm crowded with pigment-granules. ZEISS 2 m., oc. 12.
- Fig. 59. Frontal section of a fission-tail of *P. mac.*, showing groups of cells (*y*) which are probably dormant yolk glands. LEITZ obj. 3, oc. 2. (N.)
- Fig. 60. Detail drawing of a few cells of one of the groups. LEITZ obj. 7, oc. 2. (N.)
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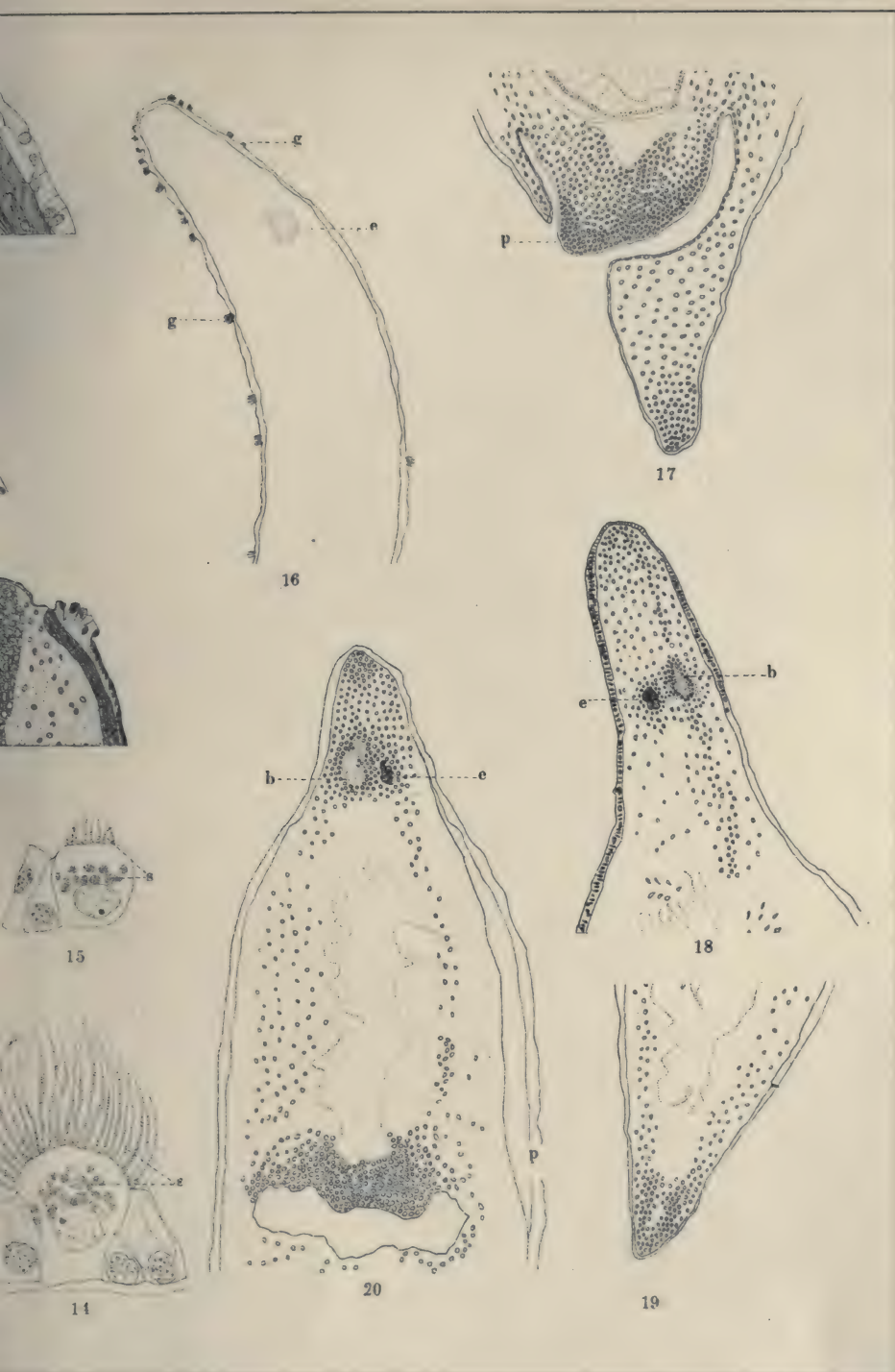






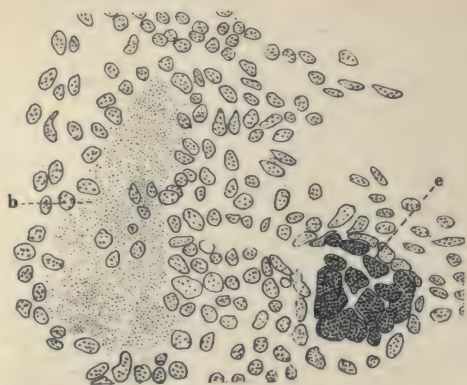




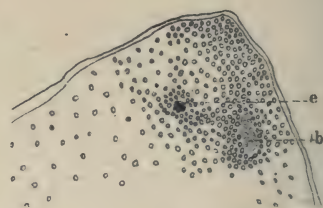




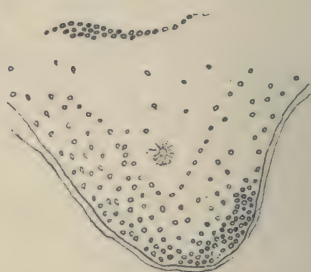




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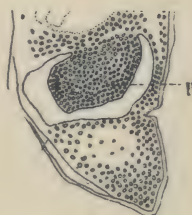
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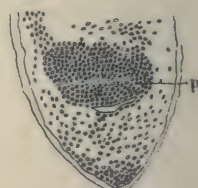
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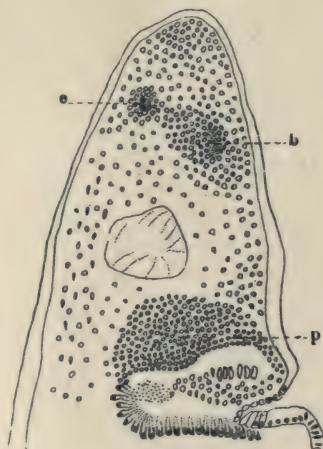
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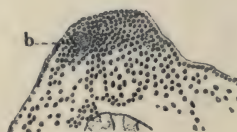
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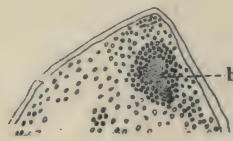
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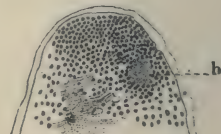
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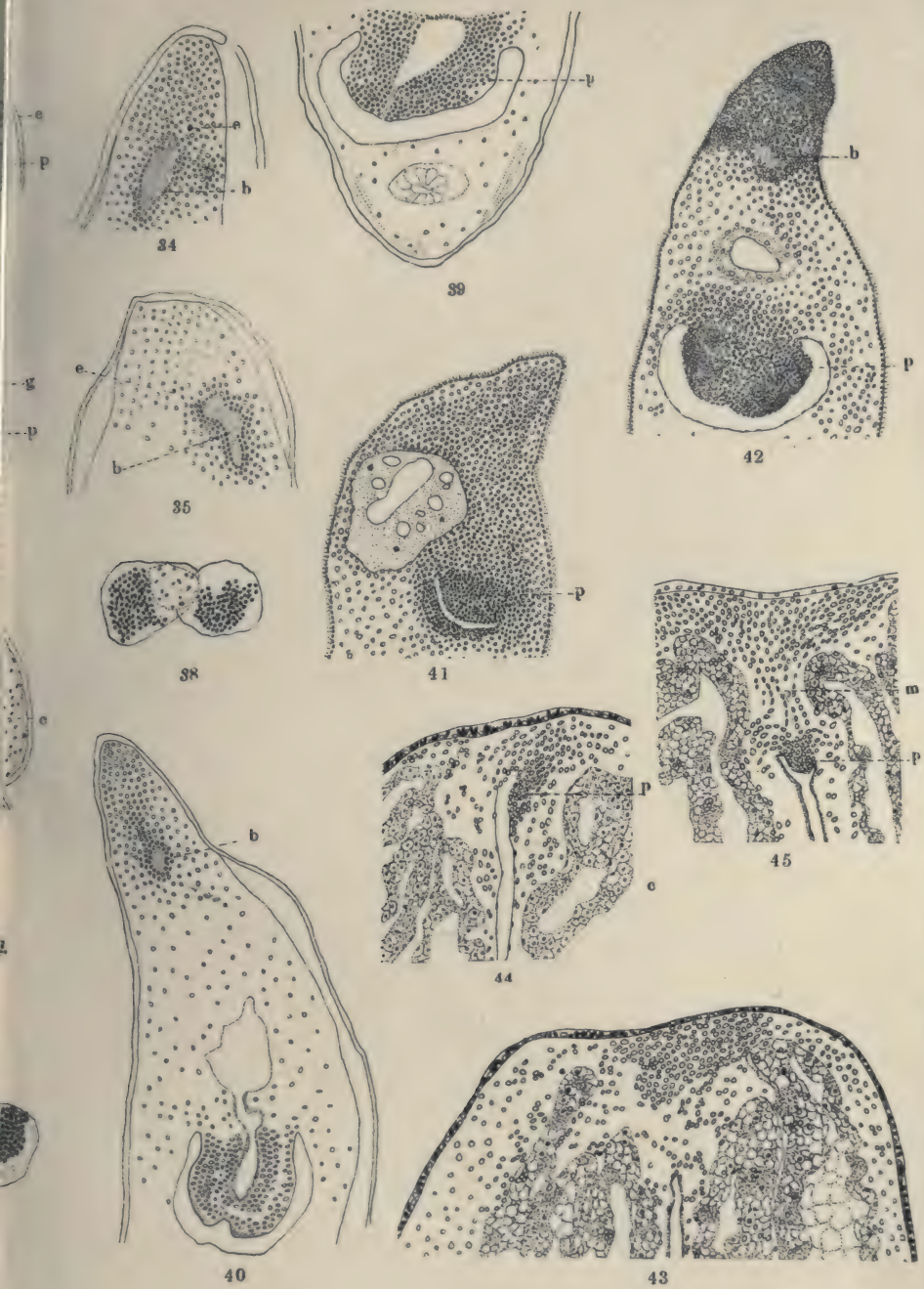
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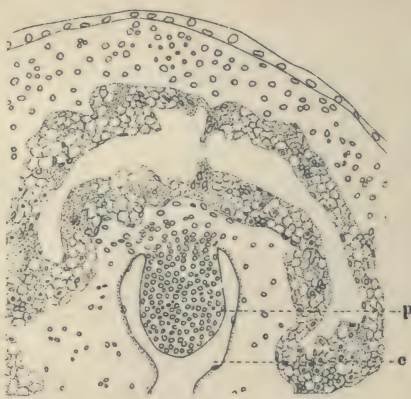


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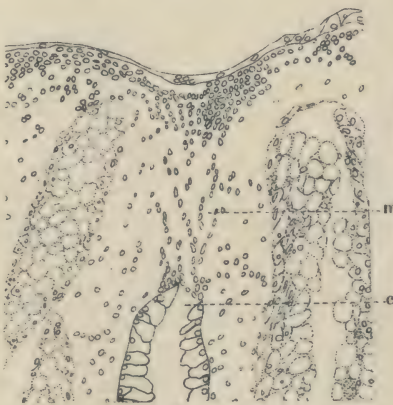




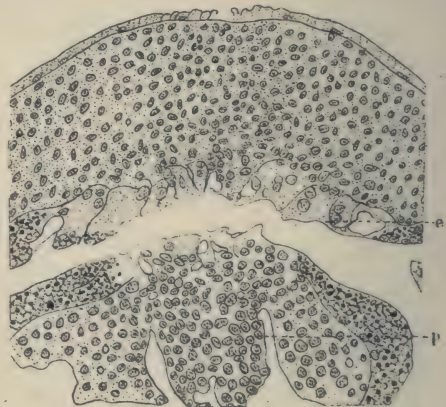
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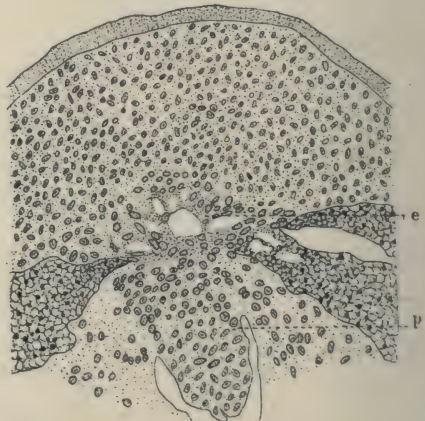
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COLOR INHERITANCE AND SEX INHERITANCE IN  
CERTAIN APHIDS

THE color changes that occur in the sexual generation of certain aphids, and the correlation of a definite color with each sex, have suggested that these insects may furnish favorable material for testing the possibility that the male and female sex characters form an allelomorphic pair and undergo segregation in gametogenesis.

In one of the goldenrod aphids all of the parthenogenetic individuals are a deep reddish brown, the males are green and the females brown, both males and females being produced by the same mother. Assuming that sex may be regarded as an inheritable character, the indications are that the parthenogenetic individual is both a sex-hybrid and a color-hybrid, green color and male sex being recessive. In the sexual generation green color becomes dominant with the male sex, brown with the female. Here correlation of color with sex, and selective fertilization, *i. e.*, only gametes containing opposite sex characters forming fertile unions, would account for the conditions observed.

In another aphid found on the star cucumber, the parthenogenetic generations consist of green and red individuals. Both red males and green females are produced by the same parthenogenetic mother which may be either green or red. Here again it appears that all of the parthenogenetic individuals must be both sex-hybrids and color-hybrids, but either color may be dominant during the parthenogenetic generations with no evident determining factor.

In an aphid which is abundant on the flower clusters and upper leaves of *Enothera biennis*, we find more complicated conditions. In the parthenogenetic generations there are two colors, a dark red and a bright green. In autumn certain red-winged mothers produce red apterous females, and other apterous red individuals produce greenish-brown males, while red females and green males come from the green mothers. The winged males are produced only by apterous mothers, the apterous females only by winged mothers. In this case all may or may not be sex-hybrids and color-hybrids.

In November, 1905, I placed sexual forms of these *Enothera* aphids on *Enothera* rosettes in the greenhouse and an abundance of eggs were laid. The eggs hatched early in March, giving both red and green young. Individuals of the two colors were isolated on *Enothera* plants protected with fine tarlatan, and the several families were kept under observation in the greenhouse until June 14, about three months from hatching. All of the members of each family remained true to the color of their egg-ancestor. The plants with the aphids were then taken to Cold Spring Harbor and planted out under tarlatan screens. Syrphid larvæ killed many of the aphids. The last of the green ones disappeared in August, while some of the red ones lived until the last of September. In no case did any individual of any one of the families deviate in color from its egg-ancestor. Sexual forms did not appear before I left Cold Spring Harbor on September 28. After returning to Bryn Mawr, about October 1, I collected both green and red parthenogenetic aphids from wild *Enotheras* and raised the males and females of the sexual generation from these. The males of green parentage are bright green like their mothers, while the females are pale green when born and gradually grow more

and more reddish until, when mature, they are a bright red, not quite so deep a red, however, as that of the red females from red mothers. The males of red parentage are red when born, but change gradually to a greenish brown, while the females are deep red like the mothers, the red being a little brighter at maturity than that of the parthenogenetic generations, but easily distinguishable from the brighter and more transparent red of the females of green parentage.

It will thus be seen that the color which comes from the winter egg holds for all of the parthenogenetic descendants, but when the sexual forms appear the males are green or greenish brown, and the females red, indicating some relation between color dominance and sex. That this relation can not be associated with different metabolic conditions in the two sexes is shown by the fact that in the star cucumber aphid, where there are both green and red parthenogenetic strains, the color conditions in the sexual generation are reversed,—the males are red and the females green.

A few preliminary experiments were made in November, 1906, in mating males and females raised from isolated mothers in the greenhouse. The matings and results were as follows, the letters showing the color characters which were visible:

1. ♀ R (red par.) × ♂ RG (red par.)  $\left\{ \begin{array}{l} a. \text{ All red.} \\ b. \text{ Red and green.} \end{array} \right.$
2. ♀ GR (green par.) × ♂ G (green par.)  $\left\{ \begin{array}{l} \text{All green.} \end{array} \right.$
3. ♀ R (red par.) × ♂ G (green par.)  $\left\{ \begin{array}{l} \text{No eggs hatched.} \end{array} \right.$
4. ♀ GR (green par.) × ♂ RG (red par.)  $\left\{ \begin{array}{l} a. \text{ One red.} \\ b. \text{ Several green.} \end{array} \right.$

Only a small proportion of the eggs hatched, but the results, though meager, indicate the possibility that color inheritance may here be Mendelian, and that a further study of it may throw light on the problem of sex inheritance.

The coloration of the sexual generation, however, shows that either one or the other of two conditions must probably exist: (1) All of the egg-ancestors and therefore all of the parthenogenetic individuals, as well as the males and females, are sex-hybrids as well as color-hybrids, and the factors which determine sex dominance also determine color dominance, possibly by virtue of some structural correlation of the two characters. (2) There are green hybrid strains which produce red females and red hybrid strains which produce greenish-brown males, while the red strains which produce red females may be pure reds and the green strains which produce green males may be pure greens.

The first of these suppositions, which alone could account for the conditions found in the star cucumber aphid, where parthenogenetic mothers of either color produce both red males and green females, and in the goldenrod aphid where the brown parthenogenetic mothers produce both green males and brown females, seems much more likely to be true for all.

In the following table the possibilities for the star cucumber aphid are shown under I., and those for the *Oenothera* aphid under I. and II. combined. (The color scheme must be reversed for the star cucumber aphid, G♀, R♂.) In both, the dominance of sometimes one color, sometimes the other in the parthenogenetic generations is a subject for investigation. It may be conditioned by the immediate ancestry of the gametes.

Gametes	Parth. Gen.	Sexual Gen.	Gametes
I { $\begin{matrix} G \text{ ♂} \\ R \text{ ♀} \end{matrix}$	$\begin{matrix} G(R) \\ \text{or} \\ R(G) \end{matrix}$	$\begin{matrix} \text{♂ } G(R) \\ \text{♀ } R(G) \end{matrix}$	$\begin{matrix} G \text{ ♂ sp.} \\ R \text{ ♀ sp.} \\ R \text{ ♀ egg} \\ G \text{ ♂ egg} \end{matrix}$
II { $\begin{matrix} G \text{ ♂} \\ G \text{ ♂} \\ R \text{ ♀} \\ R \text{ ♀} \end{matrix}$	$\begin{matrix} > G \\ > R \end{matrix}$	$\begin{matrix} \text{♂ } G & \text{—} & G \text{ ♂ sp. (?)} \\ \text{♀ } R & \text{—} & R \text{ ♀ egg} \end{matrix}$	

In the goldenrod aphid, if we consider the

parthenogenetic forms as essentially female, correlation of color with sex (B ♀ and G ♂), and selective fertilization would account for the observed relation of color to sex.

The second, and less likely but nevertheless interesting, possibility for the *Enothera* aphid involves the question whether a zygote can be pure as to the sex character, or unisexual. The chief point to be investigated by experiment, in addition to the study of color inheritance in cross-breeding, is whether in this aphid both males and females come from the parthenogenetic progeny of each egg-ancestor, or in some cases (G ♂) only males, and in others (R ♀) only females. To test this possibility it would be necessary to carry many families through from the egg to the following sexual generation, and very likely to repeat the experiment several times.

A large series of experiments in cross-breeding to test the color inheritance has been planned by the author for next year, and this note is published in the hope that some one may be interested to undertake experiments along the same line.

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# A STUDY OF THE SPERMATOGENESIS OF TWENTY-TWO SPECIES OF THE MEMBRACIDÆ, JASSIDÆ, CERCOPIDÆ AND FULGORIDÆ, WITH ESPECIAL REFERENCE TO THE BEHAVIOR OF THE ODD CHROMOSOME<sup>1</sup>

BY

ALICE M. BORING

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<sup>1</sup>A dissertation presented to the Faculty of Bryn Mawr College for the degree of Doctor of Philosophy.

## INTRODUCTION

The purpose of this investigation is to extend, to some families of the Hemiptera Homoptera, the studies of McClung, Stevens, Wilson and others on the relation of the accessory or odd chromosome to sex determination. Except for the aphids, which have been extensively worked out by Stevens ('05a, '06a), *Cicada tibi-cens* (Wilcox '95) and *Aphrophora quadrangularis* (Stevens '06b) are the only species of this group whose spermatogenesis has been previously described. This study covers eight species of the Membracidæ, six of the Jassidæ, four of the Cercopidæ and four of the Fulgoridæ.

My work was begun at the suggestion of Dr. N. M. Stevens at Woods Hole in the summer of 1905, continued under Prof. E. G. Conklin, at the University of Pennsylvania, in the year 1905-06, and completed under Dr. Stevens, at Bryn Mawr College, in the year 1906-07. To both Dr. Stevens and Professor Conklin I wish to express my appreciation of their valuable suggestions and constant help and inspiration. I wish also to thank Dr. Herbert Osborn of Columbus, Ohio; Mr. E. P. Van Duzee, of Buffalo; Mr. H. C. Barber, of New York City, and Dr. H. Skinner, of Philadelphia, for the identification of material.

## HISTORICAL REVIEW

Most of the work on the spermatogenesis of the tracheate arthropods has been done since 1890. Such studies as those of Bütschli ('71), La Valette St. George ('85), Platner ('86), Verson ('89), and Sabatier ('85) were concerned only with the formation of the spermatozoa, the arrangement of the cells of the testis into cysts, and the general mechanics of karyokinesis. The work of van Beneden ('84), Boveri ('87) and O. Hertwig ('90) on *Ascaris*, and Mark ('81) on *Limax*, turned the interest in the study of the sex cells to the chromosomes, while Weismann's daring hypothesis ('87) as to equational and reducing divisions added to the interest. By 1890, practically all investigations on spermatogenesis centered around the chromosomes in the spermatocyte divisions, and in

that year we find the first statement that one chromosome behaves differently from the others (Henking '90). Unfortunately there is the greatest confusion in the results for the next decade; but since Montgomery's suggestion ('01a) that synapsis means the conjugation of homologous maternal and paternal chromosomes, and its confirmation by Sutton's work on *Brachystola* ('00, '02, '03), there has been greater accord. As a consequence of this, certain fundamental theories are coming to rest on a firm foundation. The chromosomes are shown to keep their individuality from one cell generation to another. The real reduction in number is proved to be brought about by the joining of each paternal to a corresponding maternal chromosome in synapsis. It is found to make no difference whether the reducing or equational division comes first, but the distinction between these two divisions is constant, the one being the separating of the individual spermatogonial chromosomes, the other a simple splitting of these univalent chromosomes. In addition to this, recent work indicates that there is usually present throughout the Tracheata an odd chromosome in the spermatogonia, which behaves differently from the other chromosomes throughout its history. Still later work seems to establish the fact that this chromosome has no paternal mate, does not join any other chromosome in synapsis, divides in only one spermatocyte division, and enters only half of the spermatozoa. In some forms, a small chromosome is present as the paternal mate of this odd chromosome, but dimorphism of the spermatozoa results in either case.

The following review takes up the different observations on the Tracheata since 1890, and attempts to show how each helps to establish, or differs from, the above mentioned theories.

### *Arachnida*

Wallace ('05) finds an even number of spermatogonial chromosomes, 40, two of these being larger than the others and different in behavior. They are condensed in the spermatogonial rest stage, and take an eccentric position in the equatorial plate. They remain separate from each other in the spermatocyte growth

period and do not divide in either spermatocyte division, as the other 19 chromosomes do, thus appearing in only one quarter of the spermatozoa. Wallace concludes that all the spermatozoa degenerate except those with the two odd chromosomes.

Montgomery in *Lycosa* ('05) finds an even number of chromosomes in the spermatogonia. Two of these he calls heterochromosomes, although the only characteristic that justifies this name is that they remain condensed in the growth period. They conjugate like the other chromosomes and divide in both divisions, all of the spermatozoa receiving one-fourth of the heterochromosome tetrad.

The results of neither of these investigators agree with the more recent work on the odd chromosome in spiders and other forms. If, as Wallace states, no spermatozoa develop except those containing the two odd chromosomes and the nineteen ordinary chromosomes, the eggs must all contain only 19 chromosomes, as the spermatogonial number is 40. Suppose each egg to have 19 chromosomes; fertilization by a spermatozoön with  $19+2$  chromosomes would give all the offspring  $38+2$  ( $19+2$  in the reduced number), whether male or female; but according to Wallace's contention, the egg can have only 19; therefore it is impossible that all the spermatozoa, except those with the two odd chromosomes, degenerate. According to Montgomery, the heterochromosome in the spermatocyte is bivalent and divides in both divisions. Berry's work ('06) brings the odd chromosome in the spider into line with the odd chromosomes in other forms; it is a single chromosome in the spermatogonia, and divides in only the second division of the spermatocytes, resulting in dimorphism of the spermatozoa.

### *Myriapoda*

Blackman ('05a, '05b) finds in *Scolopendra heros* and *S. subspinipes* an uneven number of spermatogonial chromosomes. Synapsis takes place in the late anaphase of the last spermatogonial division, all of the chromosomes uniting in pairs except the odd one. The odd chromosome divides only in the second spermatocyte division. The peculiarity here is that the other chromosomes

seem to undergo their reducing division when the odd chromosome is dividing equationally, but this is only a further mark of the individuality of the chromosomes, and does not furnish any evidence against Montgomery's theory of synapsis. Medes ('05) finds a similar condition in *Scutigera forceps*.

### *Orthoptera*

Neither vom Rath ('91, '92) nor Wilcox ('95) noticed an odd chromosome in *Gryllotalpa* or *Caloptenus*, although both mention a nucleolus in the spermatocyte growth period which may be the same structure. They both insist that there are two reducing divisions; that is, two divisions that separate whole chromosomes from each other. This is probably due to a confusion in the use of the word chromosome. If we use the terminology suggested by McClung ('00), univalent chromosome in the spermatogonium, bivalent chromosome in the spermatocyte, and chromatid for each unit of the tetrad, the discrepancies in the work of vom Rath and Wilcox are cleared up. Vom Rath finds 12 spermatogonial chromosomes. In the growth period, the spireme splits into six rods, each of which forms a tetrad, or divides into four "chromosomes," as he expresses it. As he calls each chromatid a chromosome, he considers that he has two divisions which separate chromosomes from chromosomes; and therefore must be reducing; while in terms of the original spermatogonial chromosomes, one division is reducing and one equational. Wilcox falls into the same difficulty; he finds 12 spermatogonial chromosomes, and then the spireme divides into 24 "chromosomes," which form 6 tetrads. He had, in reality, 24 chromatids, and only one reducing division.

McClung ('00, '02a) has described the odd chromosome in the *Acrididæ* and *Locustidæ*. He worked on a number of forms and obtained uniform results. In the *Orthoptera*, this chromosome can be traced back into the spermatogonial rest stages. It divides only in the first spermatocyte division, giving dimorphism of the spermatozoa. In 1901, McClung suggested the theory which has since that time received substantial corroboration, that the dimor-

phism of the spermatozoa corresponds to the dimorphism of sex. McClung considers that the longitudinal division always precedes the reducing division, and thinks that this is important on account of the failure of the second polar body to be extruded in parthenogenetic eggs; but the work in the other groups of insects shows that the reducing division probably comes first as often as the equational.

Sutton's careful work ('00, '02) on *Brachystola magna* offers convincing evidence for the individuality of the chromosomes. Each pair of spermatogonial chromosomes becomes enclosed in a separate compartment of the nucleus, while the odd chromosome is in a vesicle shut completely off from the others. He suggests the application of Montgomery's theory of the union of maternal and paternal chromosomes in synapsis to Mendelian inheritance.

The observations of de Sinéty ('01) on the odd chromosome in one of the Acrididæ and in several Phasmidæ are entirely in accord with those of McClung; this chromosome divides in only one spermatocyte division, producing dimorphic spermatozoa. In one of the phasms, he finds a chromosome complex similar to that described later by McClung ('05) for *Hesperotettix*, where the odd chromosome attaches itself to one end of a tetrad, forming a hexad which divides along the transverse axis of the tetrad, thus sending the odd chromosome and two chromatids of the tetrad to one cell, and two to the other. Unfortunately de Sinéty interprets both of the spermatocyte divisions as longitudinal, but on this point he is in the minority among the workers on Orthoptera.

Baumgartner ('04), in *Gryllus domesticus*, finds the odd chromosome in a separate vesicle as Sutton did for *Brachystola*, but he finds it dividing in the second division instead of the first. Stevens ('05a) in *Stenopelmatus* and *Blatella germanica*, and Otte ('06), in *Locusta viridissima*, find that the odd chromosome divides in the second division instead of the first. Evidently there is no fixed rule as to where the odd chromosome shall divide.

Voinov ('03), Montgomery ('05) and Zweiger ('06) all hold a different view as to the valence of the orthopteran odd chromosome; but as each has studied only one species of the order, while

the work of McClung, de Sinéty, Sutton, Baumgartner and Stevens covers numerous species in several families, we have a right to question the views of these other three observers. All three hold that the heterochromosome which they describe is formed from two spermatogonial chromosomes and divides in both spermatocyte divisions.

Moore and Robinson ('05) claim that the odd chromosome in *Periplaneta americana* is only a plasmosome which dissolves before each division and is reconstructed after it.

### *Odonata*

The paper of McGill ('04) on *Anax junius* seems to show the same confusion which Wilson has discovered in Paulmier's work on *Anasa tristis*. McGill finds an even number of chromosomes in the spermatogonia, two of them small. These she identifies with the chromatin nucleolus of the rest stage and the odd chromosome, which divides in the first division and not in the second. If it could be shown that there are only 27 chromosomes in the spermatogonial plate, and that the odd chromosome is one of the larger ones, this form would fall into line with other work.

### *Lepidoptera*

The early investigators in this field, Platner ('86) and Verson ('94) paid no attention to the chromosomes. I have not been able to read Toyama's papers, but the references to them by McClung indicate that the work is not very satisfactory. Stevens ('06b) gives a few figures for two species. There are two condensed bodies throughout the growth period, which fuse in prophase like the *m*-chromosomes in *Alydus* (Wilson, '05c), and this body divides in both divisions like the equal "idiochromosomes" of *Nezara*.

### *Coleoptera*

The only work on the Coleoptera which deals with the heterochromosomes is that of Stevens ('05b and '06b) and of Nowlin ('06). Some of the beetles have an odd chromosome and others have an unequal pair in which the large member of the pair is the

maternal homologue of the odd chromosome, and the small member is the paternal mate which is lacking with the odd chromosome. In the Coleoptera, the reducing division comes first, the equational second. In this order of insects there is substantial proof of McClung's sex determination theory, as the oögonial equatorial plates have been shown to have the large chromosome, while the spermatogonial plates have the small one, and there is the same difference between the somatic plates of the males and females. The theoretical bearing of these facts will be discussed later.

### *Hemiptera*

The chromosomes in this group are so large and few in number that they have attracted many workers, but in spite of this fact, there have been greater discrepancies than in almost any other group. Henking ('90) in working on *Pyrrhocoris apterus*, was the first to notice that in one spermatocyte division, one chromosome does not divide, thus causing a dimorphism of spermatozoa. He counted 24 chromosomes in the spermatogonia, and thought that this odd chromosome had the same valence as the others. He observed a large darkly-staining nucleolus in the growth period, although he did not associate a chromatic nature with it, or connect it with the odd chromosome of the spermatocyte mitoses. He formulated no theory to account for the dimorphism of the spermatozoa.

Wilcox ('95) records that there are 12 spermatogonial chromosomes in *Cicada tibicens*, and 24 spheroidal bodies in the spermatocytes, instead of a reduced number, results similar to those on *Caloptenus femur-rubrum*.

In *Anasa tristis*, Paulmier ('99) describes two small spermatogonial chromosomes, which form first the chromatin nucleolus in the growth period, then a tetrad which divides in the first spermatocyte division, and not in the second. Because this chromosome is small and appears in only part of the spermatozoa, he regards it as degenerating chromatin. Wilson ('05c), working over the same field, finds that Paulmier has confused two bodies, inasmuch as the two small chromosomes form a tetrad and divide in

both divisions, while the odd chromosome, which divides only in the first division, is the chromatin nucleolus of the rest stage and one of the large chromosomes of the spermatogonia. He maintains that Paulmier made a mistake also in the spermatogonial number, which is always odd. Foot and Strobell ('07), by the use of smear preparations and photo-micrographs, have attempted to show that Wilson is in error in his observations on the spermatogenesis of *Anasa*. They find that the odd chromosome acts essentially like any other chromosome, is made up of two spermatogonial chromosomes and divides in both spermatocyte divisions, its only peculiarities being that it does not appear as a tetrad in prophase and occasionally divides later than the other chromosomes in metaphase. They attempt to show that the chromatin nucleolus of the rest stage is not a chromosome, but dissolves before metaphase like a plasmosome. Wilson ('07) has carefully gone over his preparations and still thinks that his former conclusions are correct. There is need of more work with smear preparations to test their reliability.

Gross ('04), in his work on *Syromastes*, apparently confuses the *m*-chromosomes with the odd chromosome much as Paulmier did. In *Pyrhocoris apterus* ('06) he finds the odd chromosome bivalent but dividing in only one spermatocyte division.

Montgomery ('01a) calls the odd chromosomes of the Hemiptera "chromatin nucleoli" and considers that they may vary in number and valence. He explains them as chromosomes on the way to disappearance during progressive evolution. His results show many discrepancies which have since been explained by Wilson ('05b and '05c).

Wilson groups the Heteroptera into three classes, those with an unequal pair of heterochromosomes, those with an odd chromosome and *m*-chromosomes, those with an equal pair of heterochromosomes. In the first class, the chromosome number in the second spermatocyte is one less than in the first spermatocyte. This is due to the fact that the conjugation of the unequal pair does not take place until after the first spermatocyte division. This is the most direct evidence yet found for Montgomery's synapsis hypothesis, for the small chromosome can be proved to be paternal,

and the large one, maternal. In the second class, the odd chromosome is homologous with the large maternal element in the unequal pair. The *m*-chromosomes are a pair, whose synapsis is delayed until just before the first spermatocyte division. The third class includes forms where there is neither an unequal pair, nor an odd chromosome, and therefore no visible dimorphism of the spermatozoa, but the fact that the equal heterochromosomes do not conjugate until after the first spermatocyte division, relates this class to the first class, and suggests that there may be a masked dimorphism, the equal heterochromosomes representing different characters, possibly, as truly as the unequal heterochromosomes where there is a visible dimorphism. Wilson cites a great deal of evidence for the individuality of the chromosomes, finding the same size relations between pairs of spermatogonial chromosomes as there are between single chromosomes in the spermatocytes. He elaborates McClung's sex determination theory, brings forward much evidence for the dimorphism of the spermatozoa, and shows that there is a corresponding dimorphism in the somatic equatorial plates of the male and female of several species of the Hemiptera heteroptera.

#### MATERIAL AND METHODS

My material was collected at Woods Hole in the summer of 1905, at Cold Spring Harbor in the summer of 1906, and at Bryn Mawr in the fall of 1906. The insects were caught in the usual sweep net, and the testes dissected out as soon as possible. Each testis consists of a group of several follicles, each attached by a separate duct to the vas deferens. The testes from the larvæ just ready for metamorphosis, and from the adults soon after metamorphosis, in most cases give all stages from the spermatogonia to the mature spermatozoa.

Before putting up material of any species, Schneider's acetocarmine proved to be a quick and efficient reagent for determining whether the testes contained all the important stages. This fixes and stains the material at the same time. The testis is put on a slide in a drop of the stain, and the cells separated by pressing down the coverglass. The preparation is made air-tight

with vaseline, and in a few minutes, the chromatin is stained a deep carmine. The entire spermatogenesis might be worked out in such preparations, the only disadvantage being that the achromatic structures are not well fixed, and the preparations are not permanent. Camera drawings made from the aceto-carmine material, compared with those from sections of material fixed in the usual reagents, show the chromosomes in the former much larger in size. (Compare Fig. 198 with Fig. 205, and 201 with 207.) This difference is largely due to shrinkage in the usual fixing fluids and alcohols. The relative sizes and positions of the structures are the same in both kinds of preparations.

If the material showed the right stages, it was put up in various fixing fluids: Gilson's mercurio-nitric, Flemming's strong chromo-aceto-osmic, Hermann's platino-aceto-osmic, and Carnoy's acetic alcohol with sublimate. The dissecting was usually done in the fixing fluid, but the small quantity of material that was dissected in physiological salt solution and immediately transferred to the fixing fluid, showed just as good fixation, as is shown by the clear outlines of all the cell structures. A few cases of poor fixation were apparently due to the long time the insects were kept in captivity, as was sometimes necessary when the material was collected several miles from the laboratory, and immediate dissection was impossible. Gilson's mercurio-nitric was the fixative used most frequently, because it gives excellent fixation of the chromatin and is a very convenient fluid to use, but nearly all material was also put in one or both of the osmic mixtures, as these give better fixation of the achromatic structures. The Gilson was used for two to six hours, the Flemming and Hermann for twelve to twenty-four hours, followed by the same length of time in running water. The Carnoy was used but little. It does not fix so well as the Gilson. Its real value is for material where an aqueous fixative cannot be used.

After fixation, the material was run through the alcohols, cleared in xylol, and embedded in paraffine with a melting point of  $52^{\circ}\text{C}$ . Most of the sections were cut  $5\ \mu$  thick, a few  $3\frac{1}{2}\ \mu$  and  $6\frac{2}{3}\ \mu$ .

Many stains were tried. The three giving most satisfactory

results were Heidenhain's iron hæmatoxylin, either without a counterstain, or with a slight tinge of orange G, thionin without a counterstain, and Auerbach's combination of acid fuchsin and methyl green. With iron hæmatoxylin, the long method gave the best results. Preparations in this stain furnish the best outlines for camera drawings, but for work in spermatogenesis, there is the disadvantage that it often stains plasmosomes and chromosomes alike. Thionin has proved a valuable stain for distinguishing between chromatic material and plasmosomes. With this material the best results are gained by leaving the slides in the stain from one to five minutes, rinsing off with water, and differentiating under the microscope with 95 per cent alcohol. The basichromatin holds the stain as a navy blue or dark purple, depending upon the material; while the plasmosome and oxychromatin either take a very pale blue, or hold no color at all. The Auerbach stain also gives differentiation between basi and oxychromatin, the odd chromosome standing out bright green in the rest stage against the pink spireme or scattered oxychromatin.

#### OBSERVATIONS

##### *Membracidæ*

In the *Membracidæ*, the testes are situated ventrally, near the anterior end of the abdomen. They are white in color, and each follicle is round. Such ripe spermatozoa as are present are found near the duct and the spermatogonia are situated on the opposite side. The rest of the follicle is filled with the intermediate stages, grouped into cysts containing cells in about the same stage. The succession of these stages is rather difficult to follow in the *Membracidæ*, because the follicles are spherical and no one longitudinal section gives all of the stages. The only way to trace the development is to find cysts with most of the cells in one stage and a few in transition to the next stage. In this way, the links between the stages can be filled in. In the eight species from which my material was obtained, the general course of development is very similar, with only here and there a striking difference. I shall there-

fore describe in detail one species, *Entilia sinuata*, and then mention the chief points of interest in the other species.

### *Entilia sinuata*

This form was found in September, at Woods Hole, on the leaves of the Golden Glow, and later near Philadelphia, on the wild sunflower.

The resting spermatogonia stain very lightly, as there are only a few basichromatin granules in the midst of much scattered oxychromatin (Fig. 1). When the cell is preparing for division, a heavy, rather darkly-staining spireme is formed with the chromatin aggregated at regular intervals along the linin (Fig. 2). A longitudinal split appears in this spireme, a slight indication of which can be seen in Fig. 2. The chromatin next becomes condensed and segmented, but these segments still retain their linin connections. The longitudinal split in each segment is also very conspicuous at this stage (Fig. 3). Condensation of the segments continues, there being first an elimination of the longitudinal split (Fig. 4), and then a shortening of the segments until they are about twice as long as broad, the form which they have as they enter the equatorial plate of the spindle (Fig. 5). They appear in the plate with their longitudinal axis at right angles to the longitudinal axis of the spindle and with the linin connections still intact. This division, therefore, is a longitudinal division, separating each chromosome into two parts along the line of the original longitudinal split, which appeared in prophase. A lateral view of the spindle in metakinesis also shows convincingly that this division is longitudinal (Fig. 6). The number of chromosomes in the spermatogonial division is 21 but it is impossible to pick out the odd chromosome. The chromosomes become so closely massed together in anaphase (Fig. 7) that one cannot tell whether the linin connections still remain intact, or the conjugation of chromosome pairs takes place here. By the time the cell division is completed, the new nuclear membrane has been formed, possibly as Conklin ('02) has suggested, by the joining together of the linin sheaths of the chromosomes after these have absorbed liquid from the cytoplasm (Fig. 8). A linin connection joining the chromo-

somes end to end is visible soon after they have lost their smooth contours (Fig. 9).

The last spermatogonial telophase is followed by a dense, darkly-staining contraction stage, which looks like a tightly wound spireme. Here the outlines of the chromosomes and their connections are entirely obliterated. The contracted mass occupies only a part of the nucleus, leaving a large clear space at one side (Fig. 10). This space appears in preparations where the fixation of other parts seems to be perfect, so it can hardly be looked upon as an artefact, as McClung ('00) at first claimed. I have used Wilson's ('05b) expression, "contraction stage" as simpler than McClung's "synizesis," for the most condensed period of "synapsis" as Moore used the term. The chromatin now goes through a series of changes comparable to those of *Anasa tristis* (Wilson '05c): (1) an early postsynapsis, with a fine spireme, much twisted on itself, still staining deeply, but filling the nucleus much more completely than in the contraction stage (Fig. 11); (2) a late postsynapsis, with the spireme filling the cell completely, less twisted, and staining unevenly (Fig. 12); (3) an early growth stage, with the spireme thicker, the basichromatin aggregated at regular intervals along the linin (Fig. 13); (4) a rest stage, where the spireme scarcely stains at all, and in the midst of the pale nucleus (in iron hæmatoxylin) there is one lens-shaped black body (Fig. 14), which, following Stevens, I shall call the odd chromosome. It is the "accessory of McClung, the "chromatin nucleolus" or heterochromosome" of Montgomery, the "chromosome spéciale" of de Sinéty, or the "heterotropic chromosome" of Wilson. From the action of similar bodies in related species, I am convinced that it must be present here in the postsynapsis and early growth stages, but the spireme stains so deeply and twists on itself so much that it hides the odd chromosome. In the succeeding stage, where the spireme becomes longitudinally split, the odd chromosome lengthens out and loses the smoothness of its outline, although not the intensity of its staining reaction (Fig. 15). The spireme next divides into ten segments, each retaining its longitudinal split (Fig. 16). Counting the odd chromosome, which remains closely applied to the nuclear membrane, there are now 11 chromatic

elements present in the nucleus. Just before the contraction stage, the spermatogonial chromosomes were joined end to end by linin connections, and out of the contraction stage there came a continuous spireme, which has passed through various stages and finally segmented. If the chromosomes conjugate end to end in the late anaphase (Fig. 8), as Fig. 9 might suggest, the longitudinal axis of the primary spermatocyte segments, or chromosomes, represents the longitudinal axis of the spermatogonial chromosomes. The presence of a massed anaphase and of the contraction stage makes it impossible to prove that this is the case here. It has, however, been proved for other forms (Sutton) and the agreement of all other steps in the process points to a possible similarity in this respect also. The 10 segments next become tetrads by the formation of transverse arms which always remain a little shorter than the longitudinal arms, and thus make it always possible to distinguish between the longitudinal and transverse axes (Figs. 17 to 19). While the tetrads and dumb-bells are forming, the odd chromosome rounds up again and becomes a lens-shaped body, still applied to the nuclear membrane (Fig. 20). It is in the dumb-bell form that the chromosomes usually enter the spindle (Fig. 24), but occasionally they are still in the form of cross-shaped tetrads (Fig. 22). This shows conclusively that the longitudinal axis of the dumb-bell is the same as the longitudinal axis of the tetrad, and that the first spermatocyte mitosis is a transverse division. That it is probably a reducing division can be shown by tracing back the development, and working out the corresponding axes: the division between the halves of the dumb-bell (Fig. 24) corresponds to a division along the lateral arms of the tetrad (Fig. 17), and that to a transverse section of the spireme segment (Fig. 16) and that to the separation of one spermatogonial chromosome from another, if we assume that each spireme segment equals two spermatogonial chromosomes joined end to end. This may be further evidence against McClung's ('00) contention that the reducing division is always the second. In the equatorial plate of the first spermatocytes the odd chromosome stands a little apart from the other 10 chromosomes, and is smaller in diameter (Fig. 21). It does not divide in the first spermatocyte division, but lags

behind the others in going toward the spindle pole (Figs. 25 and 27). The chromosomes mass together in the anaphase, so that as soon as the odd chromosome joins the others, it is no longer possible to distinguish it (Fig. 28).

The spindle fibers stand out very clearly, especially in the material fixed in Flemming or Hermann, and it is noticeable that the odd univalent chromosome is joined to only one pole by its mantle fibers, while the bivalent chromosomes are attached to both.

During the telophase the granules of a "Zwischenkörper" can be seen on some (Fig. 25) or all (Fig. 26) the spindle fibers. These show only in iron hæmatoxylin preparations which have not been extracted very thoroughly. In such preparations the centrosomes of the first spermatocyte division can also be seen (Fig. 23). They divide during the anaphase of the first division (Figs. 25 and 27) in readiness for the second division which succeeds the first without any reconstruction of the nucleus.

The chromosomes rearrange themselves (Fig. 29) into a plane at right angles to the plane of the first division, and soon form a regular equatorial plate. Half of the second spermatocytes contain 10 chromosomes (Fig. 31) and the other half 11 (Fig. 30), that is, 10 plus the odd chromosome. In the cells containing 11 chromosomes, the odd one does not differ enough in size to make it any longer distinguishable. In this division, all the chromosomes in all of the cells divide. The reasons for this conclusion are: (1) the lateral views of the metaphase (Fig. 32) never show one undivided chromosome among the other dividing ones, (2) all the chromosomes are attached by mantle fibers to both spindle poles, and (3) in the anaphase, there is never a lagging chromosome near one pole without a mate at the other pole (Fig. 33). That this division of chromosomes is at right angles to the first, that is, longitudinal and equatorial, is certainly conditioned by the formation of the spindle which is derived directly from that of the first division. The same fibers between the chromosomes and centrosomes remain intact, and as the centrosome divides, the chromosomes are pulled into an equatorial plate at right angles to the equatorial plate of the first spermatocyte division. This second division therefore corresponds to the preliminary longitudinal splitting of

the spireme in the growth period. One spermatocyte division is reducing and the other equational. In the anaphase, the chromosomes again become massed together (Fig. 34) and the nucleus is reconstructed by the formation of a nuclear membrane (Fig. 35). The "Zwischenkörper" is again noticeable in this telophase.

In the young spermatid (Fig. 36), the chromatin is still massed together and stains deeply. The spindle material remains as the "Nebenkern," as first described by v. La Valette St. George ('86) for insect spermatids. The chromatin soon scatters through the nucleus in definite clumps and it is evident that half of the spermatids contain a smooth round darkly-staining body (Fig. 37), while the other half do not (Fig. 38). Through several succeeding stages, this same fact is noticeable; *i. e.*, when the chromatin becomes more diffuse (Figs. 39 and 40), when it forms a pale network and the axial filament has grown out (Figs. 41 and 42), and even when the chromatin has begun to condense to form the head of the spermatozoön (Figs. 43 and 44). The method of determining whether this body is in only half the cells or in all is as follows: cysts of spermatids in various places were picked out and the number of cells with and without this body were counted in each cyst. In studying sections, it must be remembered that parts of some cells are in another section, so even if this body ( $x$ ) were actually present in all the cells, it would not appear in all in any one section of a cyst. On the same principle, if it were actually in only half the cells, it would appear in less than half in any one section. In Entilia, this body appears in a few less than half of the spermatids. It always takes the chromatin stains, deep blue with thionin, and green with the Auerbach. As it resembles the odd chromosome of the first spermatocyte rest stages in staining reaction and contour, and as it appears in not more than one-half of the spermatids, a condition which the odd chromosome necessarily fulfills from the fact of its not dividing in the first spermatocyte division, we seem to be justified in concluding that the body  $x$  of the spermatids is a derivative of the odd chromosome of the spermatocyte. There is nothing unusual about the formation of the spermatozoön. The "Nebenkern" forms the sheath of the axial filament (Fig. 41), the acrosome differentiates from the cytoplasm at the apex of the

head, the head forms by condensation of the chromatin (Figs. 44 to 47), passing through one rather diffuse stage (Fig. 46).

### *Vanduzea arcuata*

*Vanduzea arcuata* was found in abundance on the locust trees near Cold Spring Harbor in June. The spermatogonial plates show 17 chromosomes, varying in size (Fig. 48). It is not possible to arrange them all in pairs, but at least two large pairs are well marked ( $a_1$  and  $a_2$ ,  $b_1$  and  $b_2$ ). In the growth stage, the odd chromosome appears as a long, darkly-staining body, without a smooth contour. It is at first bent upon itself in different forms (Fig. 49), and later lies at full length along the nuclear membrane (Fig. 50), resembling the same stage in *Entilia sinuata* (Fig. 15). In the equatorial plate of the first spermatocyte division, there are 9 chromosomes, two of which are larger than the others (Fig. 51,  $a$  and  $b$ ), corresponding to the four large ones in the spermatogonial plate;  $a$  is slightly larger than  $b$  just as  $a_1$  and  $a_2$  were slightly larger than  $b_1$  and  $b_2$ . This point certainly counts as evidence that each spermatocyte chromosome represents not an indefinite segment of the spireme, but two individual spermatogonial chromosomes. The odd chromosome can be recognized by its eccentric position. Fig. 52 shows all the chromosomes but  $x$  in metakinesis, and in Fig. 53  $x$  is passing to one pole undivided. Figs. 54 and 55 show variations in the position of  $x$  in anaphase; it does not always lag behind, but may even precede the other chromosomes to the pole. The second spermatocyte equatorial plates, containing 9 and 8 chromosomes, respectively, are shown in Figs. 56 and 57. Each has one large chromosome  $a$ , one not quite so large  $b$ , and six small ones of about the same size. Fig. 56 has a ninth chromosome of intermediate size which must be the odd chromosome, as  $x$  in the first spermatocyte plate has a corresponding intermediate size (Fig. 51). All the chromosomes divide in this division, including the odd one, as is shown in all of the lateral views of the metaphase (Fig. 58) and of the anaphase (Fig. 59). Half of the spermatids contain the odd chromosome, and half do not (Figs. 60 and 61).

*Ceresa taurina*.

Three species of *Ceresa* were found near Cold Spring Harbor on the morning-glory vines and tall weeds, during the last three weeks of July. Unfortunately the chromosomes of the spermatogonial plates in all three forms are too close together to make it possible to count them. They all have the same reduced number of chromosomes and a peculiar deposition of chromatin on the nuclear membrane in the growth period. As this phenomenon is most pronounced in *Ceresa taurina*, I shall give the details for this form. In the contraction stage, the chromatin is massed at one side of the nucleus in a number of darkly-staining loops with their bases united in a dense flat chromatic plate, which stains more deeply than the loops (Fig. 62). As the loops spread through the nucleus, they stain less, making the contrast with the black plate more intense (Fig. 63). In the rest stage (Figs. 64 to 67), the reticulum does not take basic stains at all; the chromatin plate appears in various forms, sometimes continuous and sometimes broken up into two, three, or four parts. By the time a split spireme is formed, it has been almost entirely dissolved (Fig. 68), and in the prophases, no trace of it is left (Fig. 69). When these masses dissolve, the odd chromosome becomes visible as a round, smooth body (Figs. 67 and 68), which probably was concealed in the midst of the chromatic plate as far back as the contraction stage, but its presence was obscured by the similarity of its staining reaction to that of the other chromatin. As to the meaning of this deposition of chromatin on the nuclear membrane, it seems possible that it is basichromatin thrown out from the chromosome loops in the contraction stage, and that it takes no part in the further formation of the chromosomes, since it disappears before the next division. The only case at all similar which I can find in the literature is that of *Gryllus campestris* described by Voinov ('04). He claims that all the chromatin is gathered into the "corps nucleinien double," leaving the non-stainable achromatic substance spread through the nucleus, and that when the spireme forms, the chromatin is added to it again from this structure. He neglects the distinction between oxy and basichromatin, and thinks that when all

the stainable chromatin is aggregated into one body, there is no chromatin left elsewhere. The situation is much clearer if looked at from Conklin's point of view ('02): although the nucleus in the rest stage does not take basic stains, it still contains chromatin in the form of oxychromatin; this has the power of changing into basichromatin to form the chromosomes for division. The basichromatin masses of the rest stage, with the exception of the odd chromosome, which here again shows its individuality by a difference in behavior, are apparently rejected substances, which disappear without playing any further rôle in karyokinesis.

In the prophase, the odd chromosome lies close to the nuclear membrane as in the forms previously studied, and in the metaphase it has a somewhat eccentric position (Fig. 70). The chromosomes here are so nearly of the same size that it is impossible to trace any individuals from cell to cell; but the odd chromosome, by virtue of its position and its univalence, can be followed until the second spermatocytes are formed. Figs. 71 to 73 show its varying behavior in metaphase; it may either follow or precede the other chromosomes to the pole. This fact is shown also by the two anaphase figures, 74 and 75. The second spermatocyte equatorial plates show the two numbers of chromosomes 11 and 10 (Figs. 76 and 77), but the odd chromosome can no longer be distinguished from the others, either in metaphase (Fig. 78) or anaphase (Fig. 79). In all the spermatids (Fig. 80), there appears one large body (*n*) taking the basic stains, probably analogous to the body in the beetle spermatids called a chromatin nucleolus by Stevens ('06b). It is impossible to decide whether the odd chromosome in half the spermatids keeps its individuality as was observed in *Entilia* and *Van-duzea*, for all the chromatin stains deeply and in some stages is broken up into many separate masses (Fig. 80).

#### *Ceresa bubalus*

The only external difference between this species and the foregoing one is its greater size and the different angle of the prothoracic protuberances. The only difference in the spermatogenesis as can be seen by Figs. 81 to 92, is that the mass of rejected chromatin is not so conspicuous. In the bouquet stage (Fig. 81), the

plate is not nearly so large as in the same stage of *Ceresa taurina* (Fig. 63). Fig. 82 represents one of the most extreme cases of the growth stage.

#### *Ceresa diceros*

The shape and size of this species is about the same as in *Ceresa bubalus*, but the coloring is different, being brown and white, instead of uniform green. The spermatogenesis is practically the same, as Figs. 93 to 101 show, but a preparation from the testis of one could be distinguished from a preparation of the other, because the cells, chromosomes, and spindles of *C. diceros* are always smaller than those in *C. bubalus*.

#### *Atymna castanea*

This species was found on the chestnut trees exclusively, and was very abundant at the end of June and beginning of July. No spermatogonial plates in which the number of chromosomes could be counted were found. The odd chromosome appears in the rest stage as a large round body with a smooth contour and an affinity for basic stains (Fig. 102). In lateral view of the metaphase of the first spermatocyte division, it is apparent that it does not divide (Figs. 104 and 105), and in the anaphase it has the position usually characteristic of this order, between the plates of chromosomes, but nearer one pole than the other (Fig. 106). The number of chromosomes in the first spermatocyte is again 11 (Fig. 103), two of them constantly larger than the others (*a* and *b*). These two large chromosomes appear in all the second spermatocyte plates, whether they have 11 or 10 chromosomes (Figs. 107 and 108). All the spermatids contain a chromatin nucleolus (Fig. 111), as in the genus *Ceresa*. There being apparently no other basic-staining body in any of the spermatids, the odd chromosome in half of them must take part in the formation of the general reticulum like the other chromosomes.

#### *Campylenchia curvata*

*Campylenchia curvata* was found in sweepings from various weeds throughout July. The material showed all desirable stages.

Many spermatogonial plates were found, some of which it was possible to count. It seems that there must be one short period in the arrangement of the chromosomes into the plate, when they are spread further apart than at any other time. Judging from the behavior of the chromosomes of the first spermatocyte in coming into the equatorial plate, this more open stage must occur when the chromosomes are first drawn into a flat plate from their scattered position in prophase. Later as metakinesis begins and the mantle fibers pull from the two poles, the chromosomes are drawn closer together and the diameter of the plate becomes smaller. Fig. 112 shows a very clear spermatogonial plate, with 19 chromosomes. It is possible here to group the chromosomes into 9 pairs with one left over; only the two most distinct pairs are lettered,  $a_1$  and  $a_2$ , long and slender,  $b_1$  and  $b_2$ , a little shorter and thicker. The two chromosomes formed by the fusion of these pairs are designated by  $a$  and  $b$  in Fig. 114, the equatorial plate of the first spermatocyte, and in Figs. 117 and 118, the equatorial plates of the second spermatocytes. The number of chromosomes in the equatorial plates are what would be expected after finding 19 in the spermatogonia; 10 in the first spermatocytes, and 10 and 9, respectively, in the second. In the rest stages (Fig. 113),  $x$  appears as usual, but there are also present two other smaller bodies with the same staining reaction,  $m_1$  and  $m_2$ . I have called them  $m$ -chromosomes, as they have all the characteristics of Wilson's  $m$ -chromosomes in the rest stage of the Hemiptera Heteroptera ('05c); they are of equal size and they take the basic stains like the odd chromosome. As unfortunately they are not enough smaller than some of the other chromosomes to be readily distinguished in the spermatogonial plate, or to be traced through the prophase of the first spermatocyte to the spindle, it is impossible to see whether they really represent one pair whose fusion has been delayed. The odd chromosome appears as usual in metaphase (Fig. 115) and anaphase (Fig. 116) of the first spermatocyte division, and as usual is not distinguishable in the metaphase (Fig. 119) or anaphase (Fig. 120) of the second division. In the spermatids, a basic-staining body appears in half the nuclei (Figs. 121 and 122), and so must here (as in *Entilia* and *Vanduzzea*) represent the odd

chromosome, rather than the chromatin nucleolus of the other Membracidae studied.

### *Enchenopa binotata*

*Enchenopa binotata* was found throughout July at Cold Spring Harbor on the locust and wild cherry trees, on blackberry bushes and sometimes in general sweepings of weeds. Its spermatogenesis has been the most puzzling of any form studied and the following account is given tentatively, with the intention of going over the work as soon as more material can be obtained. The first facts to be noticed are that all the chromosomes appear as dumb-bells in the metakinesis of the first spermatocyte (Fig. 128), there is no lagging chromosome in the anaphase (Fig. 130), and all the second spermatocytes have 10 chromosomes (Fig. 131), the same number as the first spermatocytes. In iron hæmatoxylin preparations extracted to the same degree as in other material, no darkly-staining body appears in the rest stage, but in those extracted for a shorter time, a long twisted body appears against the pale spireme (Fig. 124). This can occasionally be traced into a stage where the spireme has segmented (Fig. 125), but never any further, as it does not assume a compact rounded shape until the other chromosomes become condensed. The question arises as to whether this body in the growth stage represents two spermatogonial chromosomes and consequently divides in both spermatocyte divisions as all bivalent chromosomes do; or whether it is univalent, analogous to most odd chromosomes in insects, but divides in the first spermatocyte division and not in the second, thus differing from all the other Hemiptera Homoptera studied and resembling most of the Heteroptera. There were a few spermatogonial plates in such a stage that it was possible to count the chromosomes, but these did not have the chromosomes as clearly spread apart as in most the other species studied. In five plates, 19 chromosomes were counted (Fig. 123) and in two, 20. One of those with 20 may, however, be deceptive; two of the chromosomes are much smaller than any in the other plates, the plate is at the surface of the section, and as  $x$  in Fig. 123 is V-shaped, it is possible that the bend of the V was cut off and the two small chro-

mosomes may really be but one. Other evidence for the univalence of one chromosome is its occasional appearance in early metaphase of the first spermatocytes when it has not yet assumed the dumb-bell shape (Fig. 129), and a few second spermatocyte metaphases where it apparently does not divide (Fig. 133). If it does not divide in the second spermatocyte division, the second spermatocyte spindle should always appear as it does in Fig. 133 rather than as in Fig. 132, unless the odd chromosome is usually in the center surrounded by the other chromosomes. That this probably is true is indicated by several cases like Fig. 135, the two anaphase groups of one second spermatocyte spindle, *a* having 9 chromosomes and *b* 10. There is a space in *a* corresponding to the chromosome marked *x* in *b*. This evidence is anything but satisfactory, but the possibility of such an exception to the general rule that the odd chromosome divides in the first spermatocyte division, is too interesting a fact to leave unmentioned. Here again one large chromosome in the first spermatocyte (Fig. 126) is represented by two in the spermatogonia (Fig. 123, *a*<sub>1</sub> and *a*<sub>2</sub>), and by one in the second spermatocyte (Fig. 131, *a*). Fig. 127 shows an occasional first spermatocyte with 11 chromosomes, implying a delay in the fusion of one pair. Here we find the chromatin nucleolus in all the spermatids (Fig. 136).

### *Jassidæ*

The testes of the Jassidæ are pale yellow in color, and therefore very easy to dissect out. The follicles are about three times as long as broad; this makes it easier to trace the development from stage to stage than in the Membracidæ. My material includes six species, four of them caught at Cold Spring Harbor in July, and the other two, *Agallia sanguinolenta* and *Phlepsius irrotatus*, at Bryn Mawr in October.

#### *Chlorotetrix unicolor* and *C. vividus*

This material was fixed and preserved as belonging to one species, but study of the sections showed two different reduced numbers of chromosomes, 11 and 9. This led to a careful com-

parison of my specimens with those in the collection at the Academy of Natural Sciences, Philadelphia. There proved to be two species, *C. unicolor* and *C. vividus*, in which the only marked difference is the width of head and thorax. Some of my specimens are slightly narrower than others, so I have probably mixed the two species, and cannot state whether the 9 chromosomes belong to *C. unicolor* or to *C. vividus*.

The resting spermatogonium has a reticulum of oxychromatin and linin and a plasmosome, which stains black in iron hæmatoxylin, but shows its achromatic nature in thionin (Fig. 137). There were no good spermatogonial plates in the material with the smaller number of chromosomes, but a lateral view of the spindle is shown in Fig. 138, and the anaphase in Fig. 139. The chromatin then passes into a contraction stage which is very dense, but contains several clear vacuoles (Fig. 140). This has a very different appearance from the contraction stage of the Membracidæ. A spireme stage follows where the chromatin again fills the nucleus and still stains deeply (Fig. 141). The odd chromosome is first visible in the rest stage (Fig. 142) where the chromatin stains least and is most scattered. It is closely applied to the nuclear membrane as was usually the case among the Membracidæ. The spireme splits longitudinally (Fig. 143), and then becomes segmented (Fig. 144). In all stages the odd chromosome can be distinguished by its small size. In the prophase of the first spermatocyte division, it can be recognized by its rounded contour; in the equatorial plate, by its eccentric position (Fig. 145); in the lateral view of the metaphase (Fig. 146), by its undivided condition; and in anaphase, by its lagging behind at one pole of the spindle (Fig. 147). In the equatorial plates of the second spermatocytes with 9 chromosomes, it can still be recognized by its small size (Fig. 149). As it divides in the second spermatocyte division, there is no indication of it in a lateral view of the metaphase (Fig. 150), or anaphase (Fig. 151). Two of the 9 chromosomes are larger than the others (*a* and *b* in Fig. 145), and they keep their individuality in the second spermatocyte (*a* and *b* in Figs. 148 and 149). In all the spermatids, there is one condensed body, which resembles the body called a chromatin nucleolus in

five species of the Membracidæ. In the early spermatid, this is the only condensed body distinguishable (Fig. 152), but later when the chromatin becomes more diffuse, it appears that half the spermatids have another smaller condensed body (Figs. 153 and 154), which is lacking in the other half. This must be the odd chromosome, observed in the same stages of three species of Membracidæ. In a still later stage, when the reticulum is arranged around a series of clear vacuoles, this difference is still to be observed; all the cells have the one large body, but only half have the small chromosome (Figs. 155 and 156). After this, both bodies disappear, the chromatin reticulum becomes slightly more condensed at first (Fig. 157), the nucleus then elongates but keeps the vacuoles (Fig. 158), and finally condenses into the head of the spermatozoön (Fig. 159). The acrosome is differentiated from cytoplasm at the apex of the head.

Fig. 160 is the spermatogonial plate of the species with the larger number of chromosomes. It contains 21 chromosomes, four larger than the others, not differing conspicuously in size among themselves ( $a_1$ ,  $a_2$ ,  $b_1$ ,  $b_2$ ). The first spermatocyte equatorial plate has 11 chromosomes, and they show the same size relation as those of the other species, two large ones and one small odd chromosome in an eccentric position (Fig. 161). This plate simply has two more chromosomes of intermediate size than the other. The second spermatocyte plates again show the two large chromosomes (Figs. 162 and 163), the total numbers being 11 and 10, instead of 9 and 8.

#### *Dicrocephala coccinea*

A few scattered individuals were found in July in general sweepings, but in August an abundance of material was obtained from the blackberry vines. The spermatogonial plates show 23 chromosomes, two larger than the others ( $a_1$  and  $a_2$  in Fig. 164). In the postsynapsis stage, the odd chromosome is not surrounded by the spireme, as has been the case in the forms described above, but it stands out distinctly by itself in the clear part of the nucleus (Fig. 165). In the rest stage, it is still of the same size and in the same position, although the nucleus grows much larger and the

chromatin becomes scattered and diffuse (Fig. 166). The first spermatocyte shows the odd chromosome as a medium-sized body, eccentric in the plate of 12 chromosomes (Fig. 167), and not dividing in metakinesis (Fig. 168). In anaphase, it lags behind the others (Fig. 169). The two large chromosomes of the spermatogonia have fused into a single large one in the first spermatocyte (*a* in Fig. 167), and this keeps its individuality in the second spermatocytes (*a* in Figs. 170 and 171). Half the second spermatocytes have 12 chromosomes, and half 11. The spermatids all have the chromatin nucleolus, and half of them the odd chromosome (Figs. 174 and 175), as in *Chlorotetrix*.

### *Diedrocephala mollipes*

This species resembles *Diedrocephala coccinea* in shape, but not in color, being bright green instead of red and green striped. Its spermatogenesis is also similar (Figs. 176 to 185), but the cells and chromosomes are smaller (cf. Fig. 177 and 167). They both have the same number of chromosomes, 12, but *Diedrocephala mollipes* has no one chromosome markedly larger than the others. The spermatids have both a chromatin nucleolus and an odd chromosome.

### *Phlepsius irrotatus*

The spermatogonial plate contains 15 chromosomes, two larger than the others (*a*<sub>1</sub> and *a*<sub>2</sub>, Fig. 186). These are represented by *a* in the first spermatocyte (Fig. 188a) and also in the second spermatocytes (Figs. 191 and 192, *a*). The growth period shows the odd chromosome (*x*) as a round body with even contour (Fig. 187). The univalent chromosome *x* has the peculiarity here that it never comes to lie in a flat plate with the other chromosomes in the first spermatocyte division, as is indicated in Fig. 189. To get all 8 chromosomes, the equatorial plate must be drawn at two different foci (Figs. 188a and 188b). The odd chromosome always precedes the others to the pole (Fig. 190), never taking the lagging position characteristic of the species previously described. We have noted that this sometimes takes place in other forms (*Vanduzee arcuata*, and the three species of *Ceresa*), but *Phlepsius* is the first form

where this position is invariable. The second spermatocytes contain 8 and 7 chromosomes (Figs. 191 and 192). The spermatids all contain the chromatin nucleolus (Figs. 195, *n*, and 196, *n*) and half of them, an odd chromosome (Figs. 195 and 196, *x*).

#### *Agallia sanguinolenta*

No spermatogonial plates were found in this form. The odd chromosome appears as usual in the growth period (Fig. 197). There are 11 chromosomes in the first spermatocyte (Fig. 198), and 11 and 10 in the second (Figs. 200 and 201). The odd chromosome does not divide in the first spermatocyte metaphase (Fig. 199), but passes to one pole after the other chromosomes in anaphase (Fig. 206). The spermatids all contain a chromatin nucleolus, and half of them, the odd chromosome (Figs. 203 and 204). Figs. 205 to 207 are drawn from aceto-carmine preparations at the same magnification as Figs. 197 to 204.

#### *Cercopidæ*

The testes of the *Cercopidæ* are situated near the posterior end of the abdomen. They are white in color, and each follicle is round, with a comparatively long duct joining it to the vas deferens. The material comprises four species, and the spermatogenesis of none of them resembles very closely that of the species studied by Stevens ('06b).

#### *Clastoptera obtusa*

This species was found on the alder at Cold Spring Harbor. The resting spermatogonium stains very lightly and has a plasmosome (Fig. 208). In preparing for division, the chromatin forms a spireme, which becomes more dense, and then segments (Fig. 209). There are 15 chromosomes in the spermatogonial equatorial plate, all of about the same size (Fig. 210). The division is longitudinal as usual (Figs. 211 and 212). After the telophase, the chromosomes soon become joined by linin connections (Fig. 213), form a compact spireme in early synapsis (Fig. 214), a dense mass in the contraction stage (Fig. 215) and a spireme loosely wound on itself in postsynapsis (Fig. 216). The odd chromosome

appears in the contraction stage distinct from the dense chromatin mass, and remains so in postsynapsis and the early growth stage (Fig. 217). It is from the first, a small, ovoid, smooth-contoured body, and still shows clearly when the spireme has segmented and the tetrads are forming (Fig. 218), and when the dumb-bells are formed (Fig. 219). It takes an eccentric position in the equatorial plate of the first spermatocyte (Fig. 220). It does not divide in the first spermatocyte division (Fig. 221), and is the last chromosome to reach the pole in the anaphase (Fig. 222). As there are 7 chromosomes, plus the odd one, in the first spermatocyte, so there are 8 in half the second spermatocytes (Fig. 223), and 7 in the others (Fig. 224). The odd chromosome behaves like the others in the second division (Figs. 225 and 226), and is not distinguishable in the spermatids, all of which have a chromatin nucleolus (Fig. 227). In the development of the spermatid, the chromatin reticulum first becomes massed on the side of the nucleus toward the axial filament (Fig. 228), and then forms a dense U, leaving the rest of the nucleus clear (Fig. 229). The nucleus then elongates, still leaving a clear space toward the apex (Fig. 230). The mature spermatozoon has a solid dense chromatic head (Fig. 231).

#### *Aphrophora quadrangularis*

This species was found on the grass and low bushes in July near Cold Spring Harbor. Originally a small quantity of material was collected and tried in aceto-carmin, as it was supposed to be the same species that Stevens ('06b) had found in Maine and described. But the reduced number of chromosomes proved to be 11 instead of 12, so material was fixed in Gilson and kept to be studied at a convenient time. The material was obtained from two distinct localities, but not kept separate. The sections showed follicles with 11 chromosomes and a few with 12. Whether this difference corresponds with the difference in locality it is unfortunately not possible to say. Another peculiarity is that the form with 12 chromosomes does not resemble, in some of its stages, the form with 11 chromosomes described by Stevens. The most important stages of the form with 11 chromosomes are shown in Figs. 232 to 242. There are 21 spermatogonial chromosomes (Fig. 232) and

11 and 10 second spermatocyte chromosomes (Figs. 238 and 239). The odd chromosome can be traced as an individual as far back as the contraction stage (Figs. 233, x, and 234, x). A plasmosome (*p*) also appears in the growth period, the thionin clearly bringing out the difference between the two. One of the 11 chromosomes is larger than the others, as is shown in Figs. 235, 238, 239. The odd chromosome does not divide in the first spermatocyte division (Figs. 236 and 237). The spermatids all contain a chromatin nucleolus (Fig. 242). A few stages of an individual with 12 chromosomes are shown in Figs. 243 to 248. This series much more nearly resembles that of the other form from Cold Spring Harbor with 11 chromosomes, than that of the form found in Maine with 12 chromosomes. The Maine form has no contraction stage (Stevens '06b, Figs. 240 to 249), while this form has a distinct one with the odd chromosome and the plasmosome outside of the spireme in the clear part of the nucleus (Fig. 243). The only possible conclusion seems to be that three species (so determined by the differences in spermatogenesis) have been up to this time grouped as one, and all called *Aphrophora quadrangularis*.

#### *Aphrophora 4-notata*

*Aphrophora 4-notata* is interesting especially in connection with *Aphrophora quadrangularis*, as being another case of difference of chromosome number within the same genus. *Aphrophora 4-notata* has 14 chromosomes for the reduced number (Fig. 250) and consequently 14 in half of the second spermatocytes (Fig. 253) and 13 in the other half (Fig. 254). The odd chromosome is present in the spireme stage (Fig. 249), and does not divide in the first spermatocyte division (Figs. 251 and 252).

#### *Fulgoridæ*

The testes of the *Fulgoridæ* are orange-colored and show through the thin white walls of the abdomen. The separate follicles are oblong. Of the four species in my material, three belong to the genus *Pæciloptera*, and one to the *Amphiscepa*, but according to the spermatogenesis, *P. bivittata* is much more like the *Amphis-*

cepa than like the other two species of *Pœcilopectera*. *P. septentrionalis* and *P. pruinosa* were found on the nettle and the other two species came from sweeping low grasses. In this material, the cells and chromosomes are large and the achromatic structures especially well preserved. The material fixed in Flemming, and stained in thionin makes some of the clearest preparations included in this study.

### *Pœcilopectera septentrionalis*

The resting spermatogonia of this form are small and stain lightly (Fig. 256). In preparation for division, a spireme is formed, each granule of which splits longitudinally (Fig. 257). The chromatic part of the spireme segments, retaining the linin connections and also an indication of the longitudinal split (Fig. 258). There are 27 chromosomes in the spermatogonial plate, two longer than the others ( $a_1$  and  $a_2$  of Fig. 259). Fig. 260 shows distinctly that this division follows the preliminary longitudinal split. After the telophase, the chromosomes become more diffuse and join into a spireme (Fig. 262). This spireme contracts into a small dense ball at one side of the nucleus (Fig. 263), and then the cell goes through a long growth period in which the diameter is at least doubled. The odd chromosome appears as soon as the spireme becomes pale enough to conceal it no longer (Fig. 264). Then a pair of *m*-chromosomes appears and a small plasmosome (Fig. 265). The plasmosome and odd chromosome both increase in size, the latter having a vacuole in the center (Fig. 266). The odd chromosome has now attained its full size, but while the cell and nucleus continue to increase, the plasmosome keeps on growing (Fig. 267). Even though it is now larger than the odd chromosome, it stains scarcely at all, while the odd chromosome and the *m*-chromosomes stain a deep blue, thus demonstrating the valuable differentiating powers of thionin. In the next stage (Fig. 268) the odd chromosome and the plasmosome are unchanged, but the spireme stains more deeply and shows a longitudinal split. The *m*-chromosomes no longer appear, they have probably become indistinguishable from the other spireme segments. The plasmosome and odd chromosome still keep the same relative size in the pro-

phase, while the tetrads are forming (Fig. 269), the plasmosome sometimes not being dissolved until after the spindle is formed (Fig. 271). There are 14 chromosomes in the equatorial plate of the first spermatocyte (Fig. 270), one of them being marked by its eccentric position, another by its large size. This large chromosome keeps its individuality in all the second spermatocytes, those with 14 chromosomes (Fig. 273), and those with 13 (Fig. 274). The odd chromosome does not divide in the first spermatocyte division (Figs. 271 and 272), but does in the second (Figs. 275 and 276). The development of the spermatid in this family is very peculiar. The nucleus stains quite deeply, so that nothing more can be made out than that there seems to be one condensed body in each spermatid (Fig. 279a). The "Nebenkern" goes through a complicated development somewhat similar at first to that described by Baumgartner ('02). First delicate fibers are formed in it (Fig. 277), then it appears as a long coiled fiber in a clear space, surrounded by a definite membrane (Fig. 278). This space becomes separated by a partition into two tubes, each containing several shorter fibers (Figs. 279a and b). These tubes and fibers both become elongated (Fig. 280). The tubes grow still longer and smaller in diameter, and at the same time twist around each other in an irregular spiral (Fig. 281a). Cross sections through different portions of these twisted tubes indicate that they must also be constricted in places (Fig. 281b). They finally become flattened, presenting some such an appearance as in Fig. 282a, and in cross section as in Fig. 282b. In this species, the chromosomes in the female somatic cells could be counted, and proved to be 28 in number (Fig. 283), there being the same two long ones that appeared in the spermatogonial plate. The significance of the even number in the female, and the odd number in the male will be pointed out in the theoretical considerations.

#### *Pœciloptera pruinosa*

*Pœciloptera pruinosa* resembles the last described form externally in every character but color, being a grayish purple instead of a pale green. The principal stages are shown in Figs. 284 to 293, the only difference being that there are two large chromosomes

instead of one, in the first spermatocyte equatorial plate (Fig. 285) and also in the second spermatocyte plates (Figs. 288 and 289). The chromatin in the spermatid nucleus does not stain so deeply, and here it can be demonstrated that there is a chromatin nucleus in all of the spermatids (Figs. 292 and 293), and the odd chromosome besides in half of them (Fig. 292). Here also the female somatic chromosome number is 28. Fig. 294 shows some of the chromosomes overlapping each other, but they are really entirely separate from one another, lying at slightly different levels; it is a late prophase stage of an egg follicle cell before the chromosomes are drawn completely into one plane.

#### *Amphiscepa bivittata*

All this material came from larvæ. The different stages are shown in Figs. 295 to 304. The spermatogonial plates contain 25 chromosomes, two pairs of long ones, one pair longer than the other (Fig. 295). In the rest stage, there are no *m*-chromosomes, but two plasmosomes are present (Fig. 296). The first spermatocyte plate shows two large chromosomes, one larger than the other (Fig. 297), corresponding to the two large pairs of the spermatogonium. The plasmosomes here persist into the metaphase (Fig. 298). The odd chromosome is quite small (Figs. 297,  $\times$ , 298,  $\times$ , 299,  $\times$ ) and does not divide in the first division. Chromosomes *a* and *b* of the first spermatocyte retain their relative sizes in the second spermatocytes, both those containing 13 chromosomes (Fig. 300), and those with 12 (Fig. 301).

#### *Pœcilopectera bivittata*

*Pœcilopectera bivittata* very closely resembles the last described species, even to the number and relative sizes of its chromosomes (Figs. 305-313). It has two plasmosomes in the growth period, and one or both of these persist in a most remarkable fashion even to the anaphase of the second spermatocyte division (Fig. 312). The size of the chromosomes and cells is greater than in *Amphiscepa bivittata*.

## THEORETICAL CONSIDERATIONS

*Individuality of the Chromosomes*

The theory of the individuality of the chromosomes was first proposed by Boveri ('88) as a result of his work on *Ascaris*. He found a constant number of chromosomes in each species, always half this number in the two maturation divisions, and the original number restored by fertilization. Every year adds to the number of species found conforming to these rules, and consequently making Boveri's theory more plausible. Beginning with Sutton's work in 1900, many species have been shown to give evidence of a more direct nature, and among these, the Hemiptera Homoptera can be classed. In the first place, it is a sign of individuality, when we are able to pick out one chromosome in every equatorial plate by some characteristic size, shape or position. This can be done for 14 out of the 22 species of Hemiptera Homoptera studied, the characteristic usually being the large size of the chromosome (see *Pæcilopectera septentrionalis*, Figs. 270, 273, 274). Secondly, all evidence that supports Montgomery's hypothesis of the union of paternal and maternal chromosomes in synapsis necessarily supports the theory of the individuality of the chromosomes. In *Pæcilopectera septentrionalis*, the large chromosome in the spermatocytes (Fig. 270) is represented in the spermatogonia (Fig. 259) by two large chromosomes. Half of the chromosomes in each spermatogonial plate must have come originally from the spermatozoön, and half from the egg. Only one large chromosome could be received from the spermatozoön, according to Fig. 270, therefore the other large one must have come from the egg. As these two large chromosomes, one paternal and one maternal, are represented by a single chromatic element in the spermatocyte, this must be formed by the union of a paternal with a maternal chromosome of the spermatogonium. Thus we see that the Hemiptera Homoptera are in accord with Montgomery's hypothesis of synapsis and reduction. In the third place, the behavior of the odd chromosome supports Boveri's theory. In the Hemiptera Homoptera, the odd chromosome can seldom be identified in the spermatogonia, but from the contraction stage to the

anaphase of the first spermatocyte, and sometimes to the metaphase of the second spermatocyte (Figs. 56 and 149) its individuality is marked. It takes the basic stains when the rest of the chromatin takes acid stains; it frequently has a smooth round contour in the early prophase, when the other chromosomes are irregular rods or tetrads; it usually is closely applied to the nuclear membrane until that is dissolved, and then keeps an eccentric position in the first spermatocyte equatorial plate; it does not divide in this division, and either precedes or follows the other chromosomes to the pole. In *Vanduzea arcuata* (Fig. 56), where it is intermediate in size, and in *Chlorotetrix* (Fig. 149), where it is the smallest chromosome, its individuality is still marked in the second spermatocyte. Finally the facts that have brought about the dropping of the old discussion about prereduction and postreduction, speak for the individuality of the chromosomes, in that they show the essential point of reduction to be the separation of each maternal chromosome from its paternal mate, and their distribution to different spermatozoa. The uselessness of insisting on prereduction or postreduction is shown within the order Hemiptera, where the odd chromosome may divide in either division; in the Heteroptera, it usually divides in the first, while in the Homoptera, the usual place of division is the second spermatocyte, but *Archimerus* and *Banassa* are exceptions in the former and *Enchenopa* in the latter.

#### *Value of the Number of Chromosomes in Taxonomy and Evolution*

McClung ('05) states that for Orthoptera, a certain number of chromosomes is characteristic for each family, the chromosome grouping marking the genus, and the relative size of the chromosomes indicating the species. Unfortunately this is not true for the Hemiptera Homoptera as the number varies within the family and even within the genus, being constant for the species only. The case of *Aphrophora quadrangularis* may make this doubtful, although it seems more probable that two or three species have previously been included under one name, than that in the same species, the reduced number should be sometimes 12 and sometimes 11, which would not accord with the simplest laws of heredity.

Montgomery has for many years endeavored to determine the stage of evolution by the number of chromosomes that a species possesses, those having few being considered higher in the scale than those with many. The chromatin nucleoli were supposed to be degenerating chromosomes as a species evolves to a higher form. But he has recently collected data from all the scattered literature, tabulated the number of chromosomes and the species, and finds that there is no such correlation ('06b). In the Hemiptera Homoptera there is no reason for considering *Vanduzea arcuata*, with 9 chromosomes, more highly evolved than *Entilia sinuata*, with 11, or *Phlepsius irrotatus*, with 8, more so than *Pæcilopectera septentrionalis*, with 14.

### *Sex Determination*

We have seen in the historical review of the work on tracheate spermatogenesis, that the most recent and reliable work all points to a dimorphism of the spermatozoa in the forms with an odd chromosome or an unequal pair of chromosomes. McClung was the first to suggest that the one characteristic that most generally divides the animal kingdom into two equal classes is sex, and that therefore, the dimorphism of sex and of spermatozoa may be causally connected. There is need of careful statistical work on the proportion of males and females among different species of insects. In general collecting, however, one gets an impression of equality in numbers. McClung's theory was a brilliant guess, which the work of Stevens and Wilson has substantiated.

The Hemiptera Homoptera furnish additional evidence for this theory. Females of many of the species were sectioned for oögonial and somatic equatorial plates. Only two furnished the desired stages, *Pæcilopectera septentrionalis* and *Pæcilopectera pruinosa*. In both the spermatogonial number is 27, the spermatozoa possessing 13 and 14 chromosomes, and the female somatic number is 28. Stevens and Wilson have shown that there is no difference between the somatic number and the unreduced number in the germ cells; in the female, both numbers are even, in the male, both are odd (or even, when a small chromosome is included). As the female somatic number in *Pæcilopectera* is even, the oögonial

number must also be even, and all the matured eggs necessarily possess the same number of chromosomes, 14. Applying Wilson's ('06b) formula for sex determination to the *Pœciloptera*, we have the following:

I Egg (14 chromosomes) + Spermatozoön (14 chromosomes)  
= Female (28 chromosomes).

II Egg (14 chromosomes) + Spermatozoön (13 chromosomes)  
= Male (27 chromosomes).

Here again it is possible to apply Castle's ('00) theory of sex as a Mendelian character, which has been so fully elaborated and applied to the case of the odd chromosome by Wilson. It involves the assumption of two kinds of eggs, male and female, as well as the two kinds of spermatozoa which are actually to be observed. It also involves the assumption of selective fertilization: an egg bearing the female determinant must be fertilized by a spermatozoön with the male determinant, while an egg bearing the male determinant must be fertilized by a spermatozoön with the female determinant. In case II of the above formula when the egg is fertilized by the spermatozoön without the odd chromosome, the sex determinant must be introduced by the egg; and as in this case, a male is produced, the eggs fertilized by a spermatozoön without an odd chromosome must bear the male determinant, and the chromosome which has disappeared in the males must be the one with the female character. So in case I, where the egg is fertilized by the spermatozoön with the odd chromosome, the spermatozoön must bear the male character and the egg the female; as this combination always results in a female, it is necessary to assume that the male character is recessive and the female dominant. The above formulæ can be extended to show these assumptions and will read thus:

I ♀ Egg (14 chromosomes) + (♂) Spermatozoön (14 chromosomes) = ♀ (♂) Female (28 chromosomes).

II (♂) Egg (14 chromosomes) + (o) Spermatozoön (13 chromosomes) = (♂) (o) Male (27 chromosomes).

This is the part of Wilson's theory that deals with the case presented by *Pœciloptera* and presumably the other Hemiptera Homoptera. The facts as far as they go are not at variance with the theory.

## SUMMARY

1 An odd chromosome is present in the spermatogenesis of 22 species of the Hemiptera Homoptera, as shown in each case by some or all of the following facts:

*a* The spermatogonia have an uneven number of chromosomes.  
*b* A dense body takes basic stains in the growth period.  
*c* One chromosome stands in an eccentric position in the first spermatocyte equatorial plate.

*d* In the metaphase of the first spermatocyte division, one chromosome does not divide, and has half the valence of the others, as shown by its spherical shape when the others are like dumbbells.

*e* In anaphase of the first spermatocyte division, one chromosome at one pole behaves differently from the others, either preceding or lagging behind.

*f* Half of the equatorial plates of the second spermatocytes contain the same number of chromosomes as those of the first spermatocytes, but half contain one less.

*g* Half of the spermatids contain a condensed body, taking basic stains, which is the odd chromosome.

2 The odd chromosome shows certain variations in behavior, either individual or specific.

*a* In the anaphase of the division where it does not divide, in some cells it may precede the other chromosomes to the poles, while in others it lags behind them.

This individual variation is a characteristic of certain species, the three species of *Ceresa* and *Vanduzea arcuata*, while most of the species studied have the odd chromosome always lagging behind, and *Phlepsius irrotatus* has it always preceding the others.

*b* In *Enchenopa binotata*, it divides in the first division, and in the second division, where it does not divide, it neither precedes nor lags behind the others.

*c* The shape of the odd chromosome in the growth period varies. It may be always spherical or ovoid with a smooth contour, as in the *Fulgoridæ*, *Cercopidæ*, *Jassidæ*, and some of the *Membracidæ*. It may be long and uneven in contour as in *Vanduzea arcuata* and *Enchenopa binotata*.

It may pass through both forms in different stages, as in *Entilia sinuata*.

3 In the spermatids of 19 species; that is, all except three of the Membracidæ, there is a chromatin nucleolus in all of the spermatids entirely independent of the odd chromosome. In seven of these species, the odd chromosome is present also in half of the spermatids, in others there is no indication of it. In the three Membracidæ without the chromatin nucleolus, *Entilia sinuata*, *Vanduzea arcuata*, and *Campylenchia curvata*, the odd chromosome is present in half of the spermatids.

4 In the genus *Ceresa*, in the contraction stage some of the basichromatin is thrown out from the chromatin loops and persists through the growth period as a chromatin deposition on the nuclear membrane and finally dissolves without apparently taking part in the formation of the chromosomes for the first spermatocyte division.

5 In three species, *Campylenchia curvata*, *Pœcilopectera septentrionalis*, and *Pœcilopectera pruinosa*, a pair of *m*-chromosomes remain condensed in the growth period.

6 The number of chromosomes has no significance for grouping species into families. In reduced number,

in the Membracidæ, 5 species have 11 chromosomes

2 species have 10 chromosomes

1 species has 9 chromosomes

in the Jassidæ, 2 species have 12 chromosomes

2 species have 11 chromosomes

1 species has 9 chromosomes

1 species has 8 chromosomes

in the Cercopidæ, 1 species has 14 chromosomes

1 species has 12 chromosomes

1 species has 11 chromosomes

1 species has 8 chromosomes

in the Fulgoridæ, 2 species have 14 chromosomes

2 species have 13 chromosomes

7 The number of chromosomes has no significance for grouping species into genera.

<i>Chlorotettrix unicolor</i> ,	11 chromosomes
<i>Chlorotettrix vividus</i> ,	9 chromosomes
<i>Aphrophora quadrangularis</i> ,	11 or 12 chromosomes
<i>Aphrophora 4-notata</i> ,	14 chromosomes
<i>Pæcilopectera septentrionalis</i> ,	14 chromosomes
<i>Pæcilopectera bivittata</i> ,	13 chromosomes

8 The number of chromosomes is constant for each species. In the case of *Aphrophora quadrangularis*, where there have been found both 11 and 12 chromosomes, probably two species are present, which have not been separated in classification.

9 The only points in the spermatogenesis in which all of the species of one family resemble each other more closely than they do the species of the other families are the appearance of some of the growth stages and the transformation of the spermatid into the spermatozoön.

10 In fourteen of the species studied, the individuality of certain chromosomes can be traced from the spermatogonium to the second spermatocyte, a pair of similar chromosomes in the spermatogonium bearing the same size relation to the other chromosomes of the equatorial plate as a single chromosome bears to the others in the first and second spermatocyte plates. In all the species, the odd chromosome can be traced as keeping its individuality from the growth period to the anaphase of the first spermatocyte division, in *Chlorotettrix* and *Vanduzeeia arcuata* to the metaphase of the second spermatocyte division, and in *Enchenopa binotata*, from the spermatogonial plate to the telophase of the second spermatocyte division.

11 In all 22 species, there is a dimorphism of the spermatozoa, which probably corresponds to the natural dimorphism of sex.

12 Two species of Fulgoridæ in which the female somatic number of chromosomes is 28, while the spermatogonial number is 27, furnish further proof for the theory of sex determination advanced by McClung, Wilson and Stevens.

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### DESCRIPTION OF PLATES.

The figures were drawn with the aid of the Zeiss-Abbe drawing camera, No. 111. Figs. 1-46 were drawn with a Leitz oil immersion obj.  $\frac{1}{3}$  and a Zeiss compensating oc. 12, Figs. 47-313 with a Zeiss apochromatic oil immersion obj. 2 mm., oc. 12. They have been reduced one-third, giving a magnification of about 1000 diameters.

#### *Abbreviations Used on Plates*

$a_1$  and  $a_2$  = one pair of spermatogonial chromosomes.

$a$  = a bivalent primary spermatocyte chromosome representing  $a_1$  and  $a_2$ .

$b_1$  and  $b_2$  = one pair of spermatogonial chromosomes.

$b$  = a bivalent primary spermatocyte chromosome representing  $b_1$  and  $b_2$ .

$m_1$  and  $m_2$  = a pair of  $m$ -chromosomes.

$n$  = chromatin nucleolus.

$p, p_1, p_2$  = plasmosomes.

$x$  = odd chromosome.

PLATE I

*Entilia sinuata* (Family Membracidae)

- Fig. 1 Spermatogonial rest stage.
- Fig. 2 Spermatogonial spireme.
- Fig. 3 Spermatogonium, segmentation of the spireme, each segment longitudinally split.
- Fig. 4 Spermatogonium, condensation of the segments of the spireme.
- Fig. 5 Spermatogonial equatorial plate, 21 chromosomes.
- Fig. 6 Spermatogonial metaphase.
- Fig. 7 Spermatogonial anaphase.
- Fig. 8 Spermatogonial telophase, formation of nuclear membrane.
- Fig. 9 Spermatogonial telophase, polar view.
- Fig. 10 First spermatocyte, contraction stage.
- Fig. 11 First spermatocyte, early postsynapsis stage.
- Fig. 12 First spermatocyte, late postsynapsis, fine spireme.
- Fig. 13 First spermatocyte, coarse spireme.
- Fig. 14 First spermatocyte, rest stage.
- Fig. 15 First spermatocyte, split spireme.
- Fig. 16 First spermatocyte, spireme divided into 11 split segments.
- Figs. 17-19 First spermatocyte, early prophase, tetrad formation.
- Fig. 20 First spermatocyte, late prophase, dumb-bell formation.
- Fig. 21 First spermatocyte, equatorial plate, 11 chromosomes.
- Fig. 22 First spermatocyte, metaphase, chromosomes still tetrads.
- Figs. 23, 24 First spermatocyte, metaphase.
- Figs. 25, 26 First spermatocyte, anaphase, centrosomes divided for the second division.
- Fig. 27 First spermatocyte, telophase.
- Fig. 28 First spermatocyte, telophase, polar view.
- Fig. 29 Rearrangement of chromosomes for the second spermatocyte division.
- Fig. 30 Second spermatocyte, equatorial plate, 11 chromosomes.



MEMBRACIDÆ

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## PLATE II

### *Entilia sinuata* (continued)

- Fig. 31 Second spermatocyte, equatorial plate, 10 chromosomes.  
Fig. 32 Second spermatocyte, metaphase.  
Fig. 33 Second spermatocyte, anaphase.  
Fig. 34 Second spermatocyte, anaphase, polar view.  
Fig. 35 Second spermatocyte, telophase.  
Fig. 36 Spermatid, first stage.  
Figs. 37, 38 Spermatids, second stage, half with  $x$ , half without.  
Figs. 39, 40 Spermatids, third stage, half contain  $x$ , half do not.  
Figs. 41, 42 Spermatids, formation of axial filament, half contain  $x$ , half do not.  
Figs. 43, 44 Spermatids, condensation of the chromatin, half contain  $x$ , half do not.  
Figs. 45, 46 Spermatids, later stages.  
Fig. 47 Mature spermatozoon.

### *Vandusea arcuata* (Family Membracidae)

- Fig. 48 Spermatogonial equatorial plate, 17 chromosomes.  
Figs. 49, 50 First spermatocyte, growth period.  
Fig. 51 First spermatocyte, equatorial plate, 9 chromosomes.  
Fig. 52 First spermatocyte, metaphase.  
Figs. 53-55 First spermatocyte, anaphase.  
Figs. 56, 57 Second spermatocytes, equatorial plates, containing 9 and 8 chromosomes, respectively.  
Fig. 58 Second spermatocyte, metaphase.  
Fig. 59 Second spermatocyte, anaphase.  
Figs. 60, 61 Spermatids, half contain  $x$ , half do not.



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PLATE III

*Ceresa taurina* (Family Membracidae)

- Figs. 62, 63 First spermatocyte contraction stage, a mass of rejected basicchromatin at the base of the loops.
- Figs. 64-66 First spermatocyte, rest stage, showing rejected basicchromatin.
- Fig. 67 First spermatocyte, rest stage, showing  $x$  in the midst of the rejected chromatin.
- Fig. 68 First spermatocyte, split spireme stage. Most of the rejected chromatin has dissolved, showing  $x$  plainly.
- Fig. 69 First spermatocyte, prophase.
- Fig. 70 First spermatocyte, equatorial plate, 11 chromosomes.
- Figs. 71-73 First spermatocyte, metaphase.
- Figs. 74, 75 First spermatocyte, anaphase.
- Figs. 76, 77 Second spermatocyte, equatorial plates, containing 11 and 10 chromosomes, respectively.
- Fig. 78 Second spermatocyte, metaphase.
- Fig. 79 Second spermatocyte, anaphase.
- Fig. 80 Spermatid, with chromatin nucleolus.

*Ceresa bubalus* (Family Membracidae)

- Fig. 81 First spermatocyte, synapsis stage, showing rejected chromatin.
- Fig. 82 First spermatocyte, rest stage, showing rejected chromatin.
- Fig. 83 First spermatocyte, equatorial plate, 11 chromosomes.
- Figs. 84, 85 First spermatocytes, metaphase.
- Figs. 86, 87 First spermatocytes, anaphase.
- Figs. 88, 89 Second spermatocytes, equatorial plates, containing 11 and 10 chromosomes, respectively.
- Fig. 90 Second spermatocyte, metaphase.
- Fig. 91 Second spermatocyte, anaphase.
- Fig. 92 Spermatid, with chromatin nucleolus.

*Ceresa diceros* (Family Membracidae)

- Fig. 93 First spermatocyte, rest stage, showing rejected chromatin.
- Fig. 94 First spermatocyte, equatorial plate, 11 chromosomes.
- Fig. 95 First spermatocyte, metaphase.
- Fig. 96 First spermatocyte, anaphase.
- Figs. 97, 98 Second spermatocytes, equatorial plates, containing 11 and 10 chromosomes, respectively.
- Fig. 99 Second spermatocyte, metaphase.
- Fig. 100 Second spermatocyte, anaphase.
- Fig. 101 Spermatid with chromatin nucleolus.

*Atymna castanea* (Family Membracidae)

- Fig. 102 First spermatocyte, rest stage.
- Fig. 103 First spermatocyte, equatorial plate, 11 chromosomes.



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PLATE IV

*Atymna castanea* (continued)

- Figs. 104, 105 First spermatocyte, metaphase.  
 Fig. 106 First spermatocyte, anaphase.  
 Figs. 107, 108 Second spermatocytes, equatorial plates, containing 11 and 10 chromosomes, respectively.  
 Fig. 109 Second spermatocyte, metaphase.  
 Fig. 110 Second spermatocyte, anaphase.  
 Fig. 111 Spermatid, with chromatin nucleolus.

*Campylenchia curvata* (Family Membracidae)

- Fig. 112 Spermatogonial equatorial plate, 19 chromosomes.  
 Fig. 113 First spermatocyte, rest stage.  
 Fig. 114 First spermatocyte, equatorial plate, 10 chromosomes.  
 Fig. 115 First spermatocyte, metaphase.  
 Fig. 116 First spermatocyte, anaphase.  
 Figs. 117, 118 Second spermatocytes, equatorial plates, containing 10 and 9 chromosomes, respectively.  
 Fig. 119 Second spermatocyte, metaphase.  
 Fig. 120 Second spermatocyte, anaphase.  
 Figs. 121, 122 Spermatids, half with  $\alpha$ , half without.

*Enchenopa binotata* (Family Membracidae)

- Fig. 123 Spermatogonial equatorial plate, 19 chromosomes.  
 Fig. 124 First spermatocyte, spireme stage.  
 Fig. 125 First spermatocyte, early prophase.  
 Fig. 126 First spermatocyte, equatorial plate, 10 chromosomes.  
 Fig. 127 First spermatocyte, equatorial plate, 11 chromosomes, occasionally found.  
 Figs. 128, 129 First spermatocytes, metaphase.  
 Fig. 130 First spermatocyte, anaphase.  
 Fig. 131 Second spermatocyte, equatorial plate, 10 chromosomes.  
 Figs. 132, 133 Second spermatocytes, metaphase.  
 Fig. 133  $\alpha$  does not divide in this division.  
 Fig. 134 Second spermatocyte, anaphase.  
 Fig. 135a and b Second spermatocyte anaphase, two plates from the same spindle, 9 chromosomes in one, 10 in the other.  
 Fig. 136 Spermatid, with chromatin nucleolus.

*Chlorotetrix unicolor* and *Chlorotetrix vividus* Family Jassidae

- Fig. 137 Spermatogonial rest stage.  
 Fig. 138 Spermatogonial metaphase.  
 Fig. 139 Spermatogonial anaphase.  
 Fig. 140 First spermatocyte, contraction stage.  
 Fig. 141 First spermatocyte, spireme stage.  
 Fig. 142 First spermatocyte, rest stage.  
 Fig. 143 First spermatocyte, split spireme stage.  
 Fig. 144 First spermatocyte, prophase.  
 Fig. 145 First spermatocyte, equatorial plate, 9 chromosomes.  
 Fig. 146 First spermatocyte, metaphase.



MEMBRACIDÆ AND JASSIDÆ

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## PLATE V

### *Chlorotetrix unicolor* and *Chlorotetrix vividus* (continued)

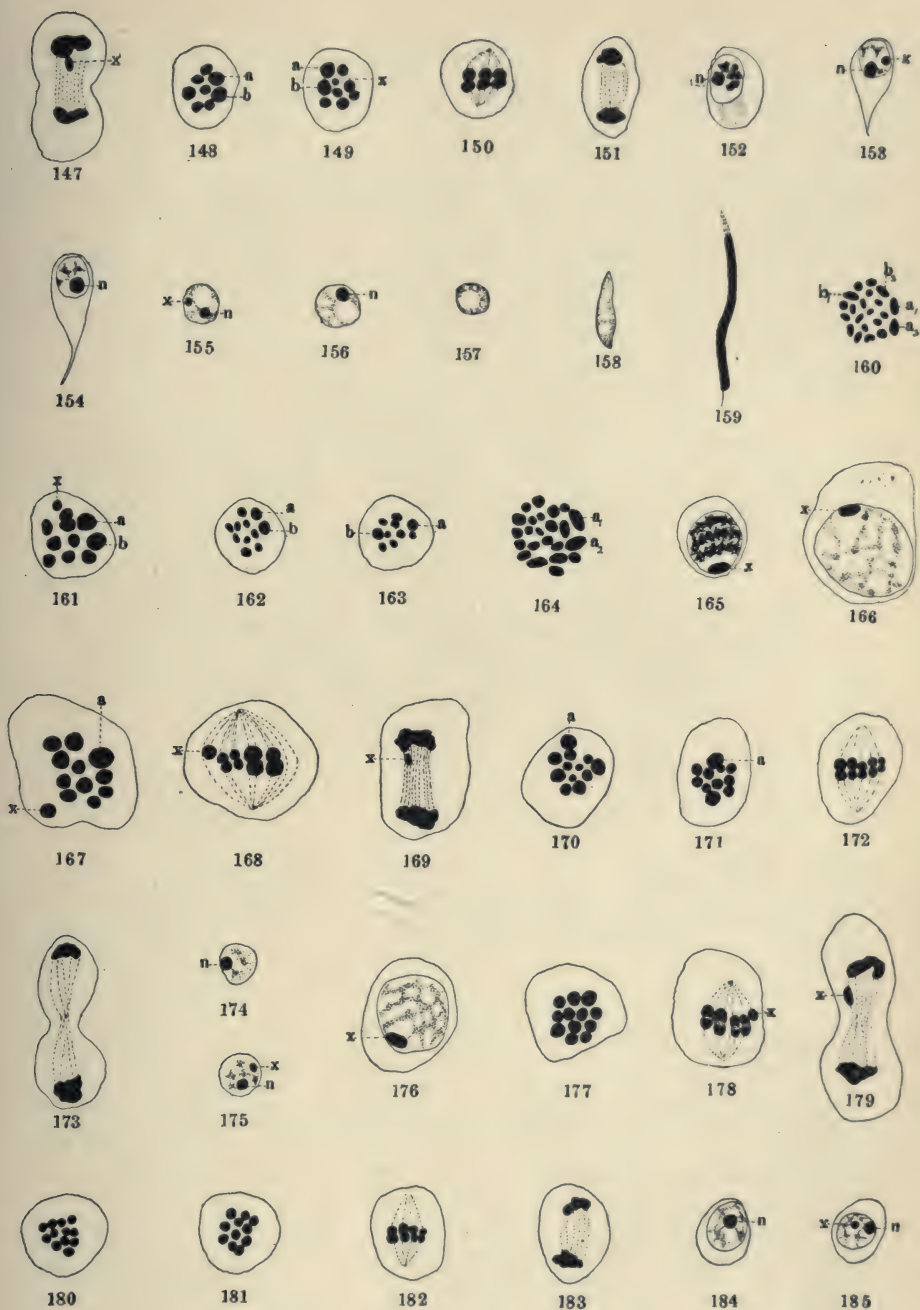
- Fig. 147 First spermatocyte, anaphase.  
Figs. 148, 149 Second spermatocytes, equatorial plates, containing 8 and 9 chromosomes, respectively.  
Fig. 150 Second spermatocyte, metaphase.  
Fig. 151 Second spermatocyte, anaphase.  
Fig. 152 Spermatid, first stage.  
Figs. 153, 154 Spermatid, second stage, half with  $x$ , half without.  
Figs. 155, 156 Spermatid, third stage, half with  $x$ , half without.  
Figs. 157, 158 Late spermatid stages.  
Fig. 159 Head of mature spermatozoön.  
Fig. 160 Spermatogonial equatorial plate, 21 chromosomes.  
Fig. 161 First spermatocyte equatorial plate, 11 chromosomes.  
Figs. 162, 163 Second spermatocytes, equatorial plates, containing 11 and 10 chromosomes, respectively.

### *Dicrocephala coccinea* (Family Jassidae)

- Fig. 164 Spermatogonial equatorial plate, 23 chromosomes.  
Fig. 165 First spermatocyte, postsynapsis stage.  
Fig. 166 First spermatocyte, rest stage.  
Fig. 167 First spermatocyte, equatorial plate, 12 chromosomes.  
Fig. 168 First spermatocyte, metaphase.  
Fig. 169 First spermatocyte, anaphase.  
Figs. 170, 171 Second spermatocytes, equatorial plates, containing 12 and 11 chromosomes, respectively.  
Fig. 172 Second spermatocyte, metaphase.  
Fig. 173 Second spermatocyte, anaphase.  
Figs. 174, 175 Spermatids, half without  $x$ , half with.

### *Dicrocephala mollipes* (Family Jassidae)

- Fig. 176 First spermatocyte, rest stage.  
Fig. 177 First spermatocyte, equatorial plate, 12 chromosomes.  
Fig. 178 First spermatocyte, metaphase.  
Fig. 179 First spermatocyte, anaphase.  
Figs. 180, 181 Second spermatocytes, equatorial plates, containing 12 and 11 chromosomes, respectively.  
Fig. 182 Second spermatocyte, metaphase.  
Fig. 183 Second spermatocyte, anaphase.  
Figs. 184, 185 Spermatids, half without  $x$ , half with.



JASSIDÆ

A. M. B. del.

PLATE VI

*Phlepsiuss irrotatus* (Family *Jassidae*)

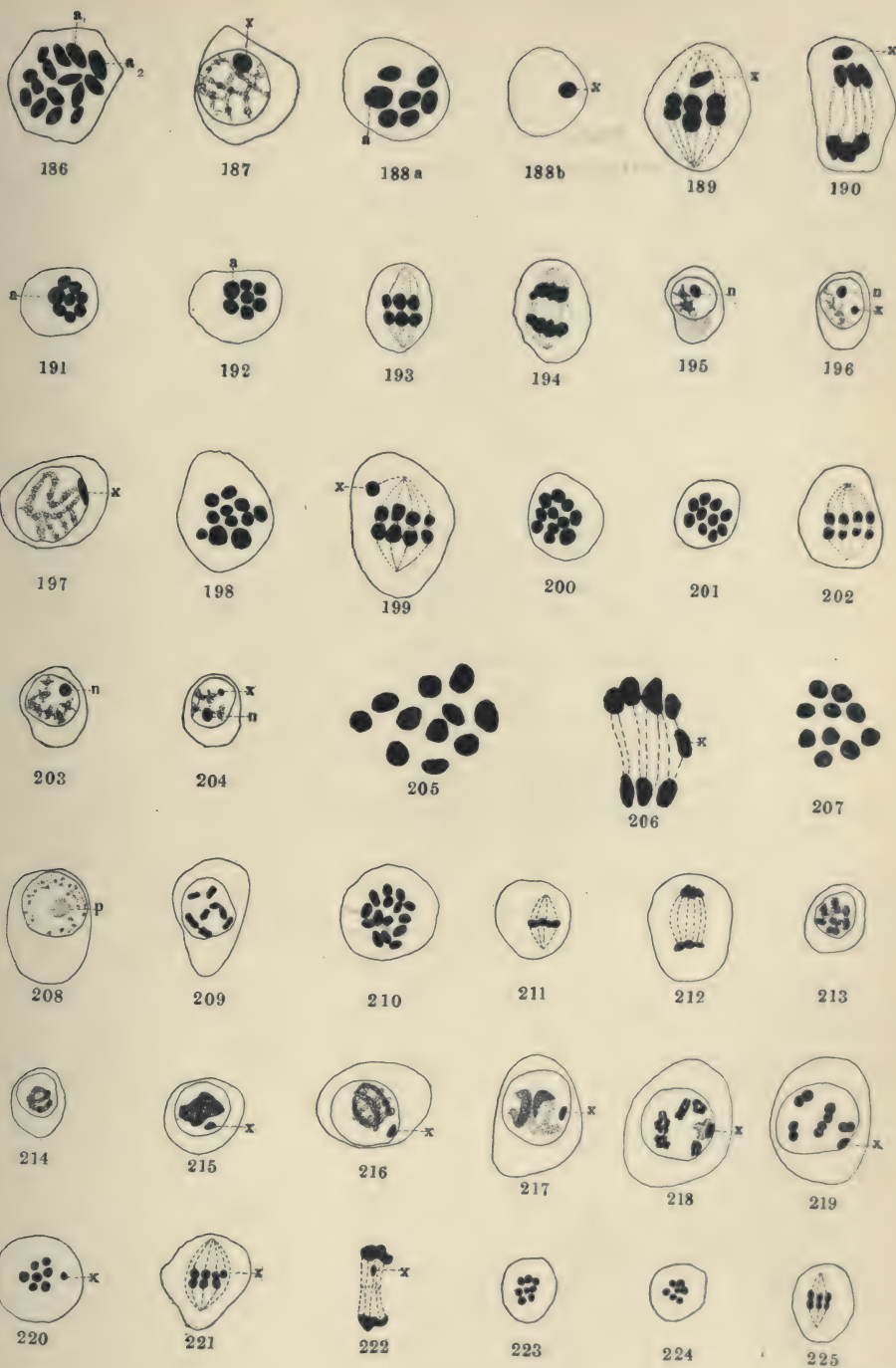
- Fig. 186 Spermatogonial equatorial plate, 15 chromosomes.  
Fig. 187 First spermatocyte, rest stage.  
Fig. 188a and b First spermatocyte, equatorial plate, and the odd chromosome  $\alpha$ .  
Fig. 189 First spermatocyte, metaphase.  
Fig. 190 First spermatocyte anaphase.  
Figs. 191, 192 Second spermatocytes, equatorial plates, containing 8 and 7 chromosomes, respectively.  
Fig. 193 Second spermatocyte, metaphase.  
Fig. 194 Second spermatocyte, anaphase.  
Figs. 195, 196 Spermatids, half without  $\alpha$ , half with.

*Agallia sanguinolenta* (Family *Jassidae*)

- Fig. 197 First spermatocyte, spireme stage.  
Fig. 198 First spermatocyte, equatorial plate, 11 chromosomes.  
Fig. 199 First spermatocyte, metaphase.  
Figs. 200, 201 Second spermatocytes, equatorial plates, containing 11 and 10 chromosomes, respectively.  
Fig. 202 Second spermatocyte, early anaphase.  
Figs. 203, 204 Spermatids, half without  $\alpha$ , half with.  
Fig. 205 First spermatocyte, equatorial plate, aceto-carmin preparation.  
Fig. 206 First spermatocyte, anaphase, aceto-carmin preparation.  
Fig. 207 Second spermatocyte, equatorial plate, aceto-carmin preparation.

*Glastoptera obtusa* (Family *Cercopidae*)

- Fig. 208 Spermatogonial rest stage.  
Fig. 209 Spermatogonial prophase.  
Fig. 210 Spermatogonial equatorial plate, 15 chromosomes.  
Fig. 211 Spermatogonial metaphase.  
Fig. 212 Spermatogonial anaphase.  
Figs. 213, 214 First spermatocyte, early synapsis.  
Fig. 215 First spermatocyte, contraction stage.  
Fig. 216 First spermatocyte, pöstsynapsis stage.  
Fig. 217 First spermatocyte, spireme stage.  
Fig. 218 First spermatocyte, early prophase, tetrad formation.  
Fig. 219 First spermatocyte, prophase, dumb-bell formation.  
Fig. 220 First spermatocyte, equatorial plate, 8 chromosomes.  
Fig. 221 First spermatocyte, metaphase.  
Fig. 222 First spermatocyte, anaphase.  
Figs. 223, 224 Second spermatocytes, equatorial plates containing 8 and 7 chromosomes, respectively.  
Fig. 225 Second spermatocyte, metaphase.



JASSIDÆ AND CERCOPIDÆ

A. M. B. del.

PLATE VII

*Clastoptera obtusa* (Continued)

- Fig. 226 Second spermatocyte, anaphase.  
Fig. 227 Early spermatid, with chromatin nucleolus.  
Fig. 228 Spermatid, formation of axial filament.  
Figs. 229, 230 Later spermatids.  
Fig. 231 Mature spermatozoön.

*Aphrophora quadrangularis* with 11 chromosomes (Family Cercopidæ)

- Fig. 232 Spermatogonial equatorial plate, 21 chromosomes.  
Fig. 233 First spermatocyte, contraction stage.  
Fig. 234 First spermatocyte, spireme stage.  
Fig. 235 First spermatocyte, equatorial plate, 11 chromosomes.  
Fig. 236 First spermatocyte, metaphase.  
Fig. 237 First spermatocyte, anaphase.  
Figs. 238, 239 Second spermatocytes, equatorial plates, containing 11 and 10 chromosomes, respectively.  
Fig. 240 Second spermatocyte, metaphase.  
Fig. 241 Second spermatocyte, anaphase.  
Fig. 242 Spermatid, with chromatin nucleolus.

*Aphrophora quadrangularis* with 12 chromosomes (Family Cercopidæ)

- Fig. 243 First spermatocyte, contraction stage.  
Fig. 244 First spermatocyte, equatorial plate, 12 chromosomes.  
Fig. 245 First spermatocyte, metaphase.  
Fig. 246 First spermatocyte, anaphase.  
Fig. 247 Second spermatocyte, equatorial plate, 12 chromosomes.  
Fig. 248 Second spermatocyte, anaphase.

*Aphrophora 4-notata* (Family Cercopidæ)

- Fig. 249 First spermatocyte, spireme stage.  
Fig. 250 First spermatocyte, equatorial plate, 14 chromosomes.  
Fig. 251 First spermatocyte, metaphase.  
Fig. 252 First spermatocyte, anaphase.  
Figs. 253, 254 Second spermatocytes, equatorial plates, containing 14 and 13 chromosomes, respectively.  
Fig. 255 Second spermatocyte, anaphase.



CERCOPITHECÆ

A. M. B. *del.*

PLATE VIII

*Pæcilopectera septentrionalis* (Family Fulgoridæ)

- Fig. 256 Spermatogonial rest stage.  
Fig. 257 Spermatogonial split spireme.  
Fig. 258 Spermatogonium, spireme segmented and condensed, segments split.  
Fig. 259 Spermatogonial equatorial plate, 27 chromosomes.  
Fig. 260 Spermatogonial metaphase.  
Fig. 261 Spermatogonial anaphase.  
Fig. 262 First spermatocyte, early synapsis stage.  
Fig. 263 First spermatocyte, contraction stage.  
Fig. 264 First spermatocyte, spireme stage.  
Figs. 265, 267 First spermatocyte, rest stages, growth in size of nucleus and cell.  
Fig. 268 First spermatocyte, split spireme stage.  
Fig. 269 First spermatocyte, prophase, tetrad formation.  
Fig. 270 First spermatocyte, equatorial plate, 14 chromosomes.  
Fig. 271 First spermatocyte, metaphase.  
Fig. 272 First spermatocyte, anaphase.  
Figs. 273, 274 Second spermatocytes, equatorial plates, containing 14 and 13 chromosomes, respectively.  
Fig. 275 Second spermatocyte, metaphase.  
Fig. 276 Second spermatocyte, anaphase.  
Figs. 277, 278 Spermatids, formation of fibers in the "Nebenkern."  
Fig. 279a Spermatid, "Nebenkern" separated by a partition into two tubes.  
Fig. 279b Cross section of "Nebenkern" structure as in 279a.  
Fig. 280 Spermatid, elongation of fibers and tubes.  
Fig. 281a Spermatid, irregular spiral of twisted tubes.  
Fig. 281b Cross sections of tubes of 281a.  
Fig. 282a Spermatid, further twisting and flattening.  
Fig. 282b Cross section of 282a.  
Fig. 283 Female somatic equatorial plate, 28 chromosomes.



FULGORIDÆ

A. M. B. del

PLATE IX

*Pæcilopectera pruinosa* (Family Fulgoridæ)

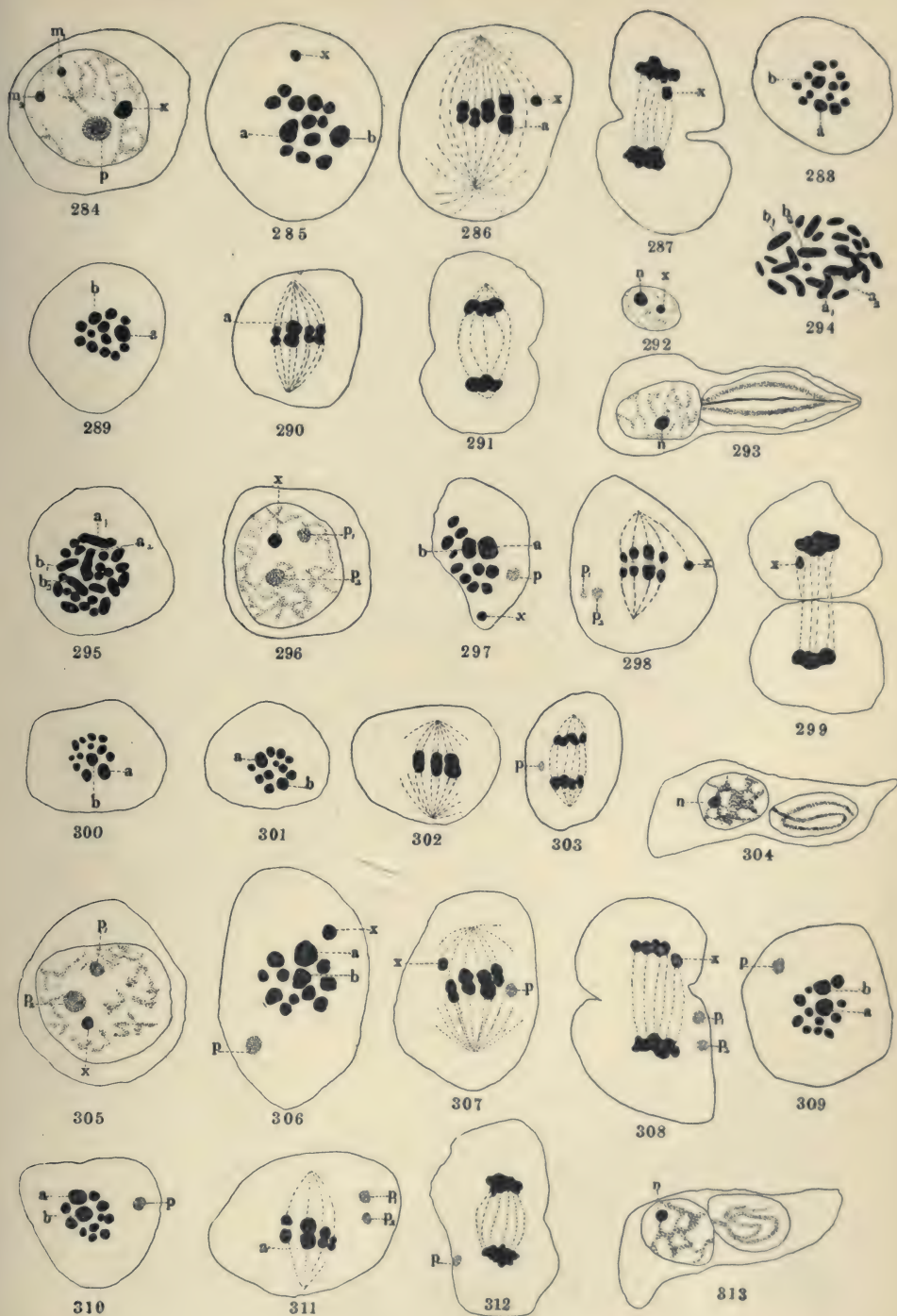
- Fig. 284 First spermatocyte, rest stage.  
Fig. 285 First spermatocyte, equatorial plate, 14 chromosomes.  
Fig. 286 First spermatocyte, metaphase.  
Fig. 287 First spermatocyte, anaphase.  
Figs. 288, 289 Second spermatocytes, equatorial plates, containing 14 and 13 chromosomes, respectively.  
Fig. 290 Second spermatocyte, metaphase.  
Fig. 291 Second spermatocyte, anaphase.  
Figs. 292, 293 Spermatids, half with  $\alpha$ , half without.  
Fig. 294 Female somatic equatorial plate, 28 chromosomes.

*Amphiscepa bivittata* (Family Fulgoridæ)

- Fig. 295 Spermatogonial equatorial plate, 25 chromosomes.  
Fig. 296 First spermatocyte, rest stage.  
Fig. 297 First spermatocyte, equatorial plate, 13 chromosomes.  
Fig. 298 First spermatocyte, metaphase.  
Fig. 299 First spermatocyte, anaphase.  
Figs. 300, 301 Second spermatocytes, equatorial plates, containing 13 and 12 chromosomes, respectively.  
Fig. 302 Second spermatocyte, metaphase.  
Fig. 303 Second spermatocyte, anaphase.  
Fig. 304 Spermatid.

*Pæcilopectera bivittata* (Family Fulgoridæ)

- Fig. 305 First spermatocyte, rest stage.  
Fig. 306 First spermatocyte, equatorial plate, 13 chromosomes.  
Fig. 307 First spermatocyte, metaphase.  
Fig. 308 First spermatocyte, anaphase.  
Figs. 309, 310 Second spermatocytes, equatorial plates, containing 13 and 12 chromosomes, respectively.  
Fig. 311 Second spermatocyte, metaphase.  
Fig. 312 Second spermatocyte, anaphase.  
Fig. 313 Spermatid.



FULGORIDÆ

A. M. B. del.



## FURTHER STUDIES ON THE PARTHENOGENETIC DEVELOPMENT OF THE STARFISH EGG.

D. H. TENNENT.

The results of investigations described in an earlier paper<sup>1</sup> led me to the view that possibly a conjugation of egg and sperm chromosomes, similar to that apparently occurring in starfish eggs that had been treated with CO<sub>2</sub> and subsequently fertilized, might be found to occur in normally fertilized eggs.

As I suggested in the paper mentioned, it would be necessary, in order to settle the question raised, to reexamine the normal fertilization and cleavage stages or to make a study of the formation of the germ cells in the starfish.

This paper deals with observations made in accordance with this plan and with some further observations made on starfish eggs developing as a result of treatment with CO<sub>2</sub>. The material for the investigation was obtained while I was occupying a room at the Marine Biological Laboratory, Woods Hole, during a portion of the summer of 1906.

I found, soon after beginning a study of fertilized starfish eggs, that the equatorial plate of the first cleavage spindle contained, with variations which I shall mention later, in eggs from some individuals 18 chromosomes, and in eggs from other individuals 36 chromosomes. I have as yet been unable to correlate this difference in the number of chromosomes with the common starfishes of the Woods Hole region, *Asterias forbesii* and *Asterias vulgaris*,<sup>2</sup> although it is probable that such a relationship will be established.

The study of the fertilized eggs proved puzzling, and it was not until I had made an investigation of the spermatogenesis of *Asterias vulgaris* and a reexamination of eggs developing parthenogenetically after treatment with CO<sub>2</sub> that I was able to find a solution for the problem under consideration.

<sup>1</sup> "Studies on the Development of the Starfish Egg," D. H. Tennent and M. J. Hogue, *Journal of Experimental Zoölogy*, Vol. III. (1906).

<sup>2</sup> Clark, "The Echinoderms of the Woods Hole Region," Bull. U. S. F. C., Vol. XXII. (1902), pp. 553-554.

Inasmuch as the basis of my interpretation lies in facts observed during the study of the male germ cells, I shall first present a brief account of these observations.

#### THE SPERMATOGENESIS OF *ASTERIAS VULGARIS*.

In well-preserved stronger Flemming material stained in iron-hæmatoxylin the spermatogonia show 18 chromosomes, these all having a slightly constricted or dumb-bell form (Fig. 1). The chromosomes are either straight or slightly bent.

The chromosomes of the primary spermatocytes are nine in number and have at first a distinct dumb-bell form. A precocious longitudinal splitting soon gives them a V or looped form which may be seen in horizontal sections of the equatorial plate (Fig. 2).

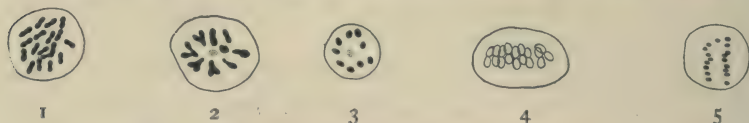


FIG. 1. Equatorial plate of spermatogonial mitosis.  
FIG. 2. Spermatocyte of the 1st order.  
FIG. 3. Spermatocyte of the 2d order. Polar view.  
FIG. 4. Spermatocyte of the 2d order. Metaphase.  
FIG. 5. Second spermatocyte division.

The secondary spermatocytes contain nine chromosomes (Figs. 3 and 4). In the second maturation mitosis these appear to be divided transversely (Fig. 5), giving nine as the reduced germ-cell number.

#### STUDIES ON EGGS.

After noting the difference in the somatic number of chromosomes in the different lots of eggs sectioned, it became evident that it was desirable to have a set of material in which the eggs from one individual had been treated in three different ways, namely, one set fertilized with sperm, another set treated with  $\text{CO}_2$  and a third set treated with  $\text{CO}_2$  and subsequently fertilized. I succeeded in obtaining one lot of material of this nature. I shall record my observations in the order of the above statement.

##### (a) *Observations on Fertilized Eggs.*

In a successful preparation, the section being sufficiently thick to show the greater number of the chromosomes of the equa-

torial plate of the first segmentation spindle in their entirety (Fig. 6), the chromosomes are seen to be of a dumb-bell form, some straight, some slightly bent, and lying with their long axis placed transversely to the spindle fibers. Their number, as may be seen from the figure, would lead one to suspect 36 as the somatic number, but owing to the fact that some of the chromosomes are cut, this may not be stated with certainty.

In an especially fortunate section passing symmetrically through the long axis of the spindle, it is seen that the chromosomes have been split longitudinally and drawn out as somewhat slender rods. In drawing this figure I have shown only the chromosomes and parts of chromosomes lying within a short

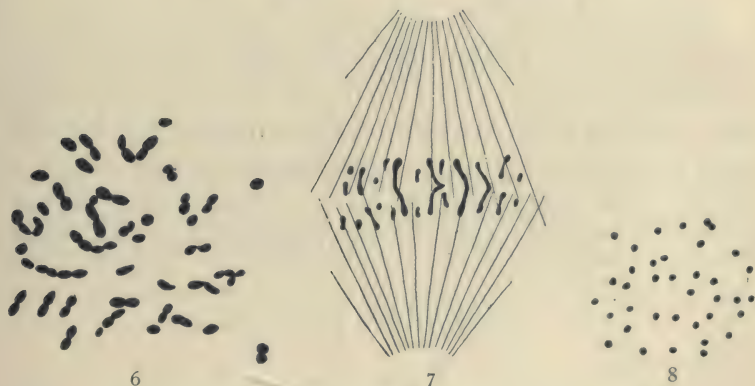


FIG. 6. Equatorial plate 1st segmentation fertilized egg. Polar view.

FIG. 7. First segmentation fertilized egg. Anaphase.

FIG. 8. First segmentation fertilized egg. Section through chromosomes as they are drawn out in anaphase.

focal range, inasmuch as it would have complicated the figure so greatly as to make it unintelligible had the chromosomes lying toward the opposite side of the spindle been added. In this section again, the full number of chromosomes could not be counted with certainty.

Finally, in a section passing transversely through the spindle of an egg in the same stage of division as that from which Fig. 7 was drawn, it is shown conclusively that the somatic number of chromosomes in this lot of fertilized eggs is 36.

*(b) Observations on Eggs Treated with CO<sub>2</sub>.*

Due precautions were of course taken to avoid chance fertilization. The control showed freedom from segmenting eggs.

In the sections of these eggs it was even more difficult than in the fertilized eggs to determine accurately the number of chromosomes. Sections thick enough to contain all of the chromosomes were unintelligible. Thinner sections were likewise of



FIG. 9. *a-b*. Sections through same equatorial plate CO<sub>2</sub> egg.

little value. Fig. 9 shows all of the chromatic material contained in the equatorial plate as demonstrated in two sections of this egg. The impossibility of stating with any reasonable degree of accuracy the number of chromosomes involved is evident

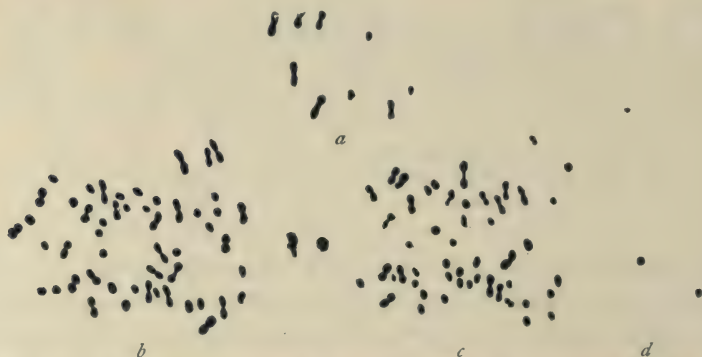


FIG. 10. *a-d*. Four longitudinal sections through 1st segmentation spindle, late anaphase. CO<sub>2</sub> egg. Spindle fibers omitted.

to any observer. Nor is the situation appreciably relieved by the examination of longitudinal sections of the spindle in later anaphase.

In such sections as those shown in Fig. 10, *a-d*, a cursory

examination would lead the observer to believe that the number of chromosomes is fully as great as that in the fertilized eggs. This view might be supported by the fact that many of the chromosomes show a form similar to that possessed by those of the fertilized eggs.

Closer examination of the position and arrangement of the chromosomes in these sections reveals the fact that the bodies have been sectioned. The imaginary superposition of one figure upon the other lends credence to such an idea.

For these reasons I have been unable to determine the number of chromosomes by actual count. The number I believe to be 18, a statement for which I shall give my reasons later.

(c) *Eggs Treated with CO<sub>2</sub> and Subsequently Fertilized.*

Sections of these eggs agree with figures that I have already published. The eggs were fertilized and underwent segmentation, the CO<sub>2</sub> simply retarding the rate of development.

OBSERVATIONS ON OTHER STARFISH EGGS TREATED WITH CO<sub>2</sub>.

I succeeded in obtaining one lot of eggs which developed after treatment with CO<sub>2</sub>, that contain in all cases only 9 chromosomes. I was unable to obtain a ripe male at the time and so can give no facts as to the fertilization of these eggs.

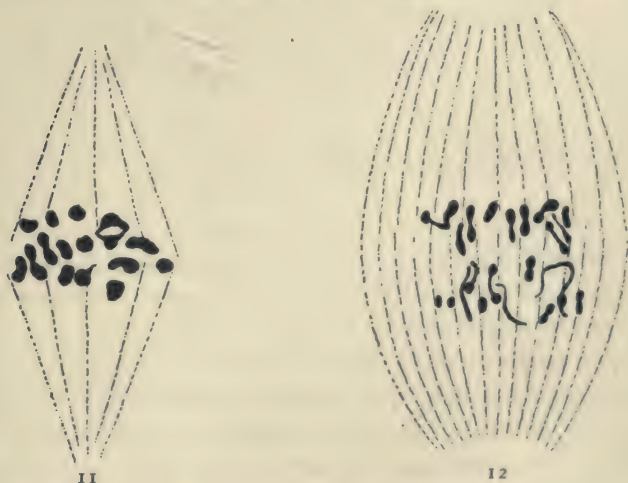


FIG. 11. First segmentation CO<sub>2</sub> egg. Early anaphase.  
FIG. 12. First segmentation CO<sub>2</sub> egg. Later anaphase.

The individual from which I obtained the eggs I identified as *Asterias forbesii* although it will be noted that the number of chromosomes agrees with the germ-cell number in my spermatogenesis material of *Asterias vulgaris*.

In these eggs, as in the other CO<sub>2</sub> eggs described, the form of the chromosomes is irregular but owing to the smaller number may be counted readily. The equatorial plate, the daughter plates, etc., all show the same number, — *i. e.*, nine (Figs. 11 and 12).

Fig. 13 shows the extremely irregular form assumed by the chromatic material in anaphase and explains the reasons for the complexity exhibited by sections such as those from which Fig. 10 was drawn.

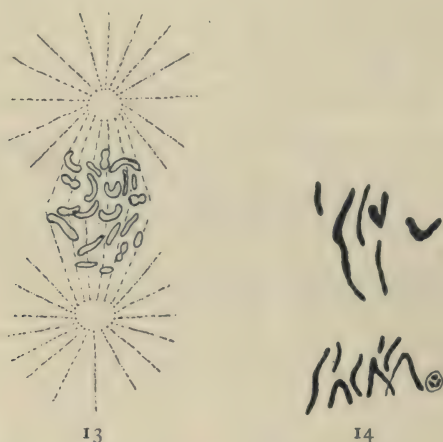


FIG. 13. First segmentation CO<sub>2</sub> egg. Anaphase.

FIG. 14. First segmentation CO<sub>2</sub> egg. Late anaphase. One chromosomal vesicle.

As shown in Fig. 13 this chromatic material at this stage is in the form of greatly twisted threads. A single section may cut the thread in several places.

In later anaphase these threads are drawn out, passing through a variety of changes and at last are embodied in chromosomal vesicles which unite to form the daughter nucleus (Fig. 14).

Clearly then, this egg with its oöcyte number of 9 chromosomes does not exhibit the phenomenon of "autoregulation."

As I have already stated, owing to my inability to make an accurate count, I have not been able to show this to be true in the case of the egg whose reduced number of chromosomes is 18. That the behavior of both eggs is probably similar will be granted by most readers.

#### GENERAL CONSIDERATIONS.

These observations show conclusively that in the fertilized egg there is no conjugation of maternal and paternal chromosomes as individuals, at the time when I thought that such a union might take place.

The facts that I have given, namely, that the reduced number of chromosomes in the male germ cell of one form is 9, that the reduced number of chromosomes in one egg is 18, and that the number in fertilized eggs is 18 and 36 is sufficient proof.

After the examination of many lots of fertilized eggs I became convinced that 18 and 36 were not constant as somatic numbers. Small variations, such as differences of one or two, might be laid to error in counting. A constant greater variation that I have found hardly seems due to the same cause.

A possible interpretation of such a greater variation is of interest.

In one lot of eggs the number 27 seems constant. In this lot I have never been able to count as many as 36 chromosomes.

Such a number, (27), is readily explained on the supposition that an egg containing 18 chromosomes has been fertilized by a spermatozoan containing 9, or that an egg with 9 has been fertilized by a spermatozoan containing 18. The result in either case would be a somatic number of 27.

Now, accepting the interpretation of synapsis as the conjugation of homologous maternal and paternal chromosomes; we shall have at the conclusion of synapsis a reduced number of 18. That is, nine pairs or nine bivalent chromosomes and nine univalents which had been unable to find mates.

Such eggs, if they were fertilized by a spermatozoan containing 18 chromosomes should give rise to individuals with a somatic number of 36, or, uniting with a spermatozoan with 9 chromosomes should retain a somatic number of 27.

If the theory of the individuality of the chromosomes is correct and the interpretation of synapsis well founded, experiments in hybridization with favorable forms ought to prove the truth of such an explanation. The starfish, owing to our inability to raise adults from the egg and to the extremely small size of its chromosomes, does not seem promising for such an investigation.

#### SUMMARY.

1. The reduced number of chromosomes in the male germ cells of *Asterias vulgaris* is 9.

2. Fertilized starfish eggs contain as a somatic number 18 and 36 chromosomes, the difference possibly to be correlated with *Asterias vulgaris* and *Asterias forbesii*.

3. Eggs caused to develop parthenogenetically show one half the somatic number of chromosomes.

4. No conjugation of individual chromosomes takes place in fertilized eggs immediately before the first segmentation.

5. A possibly hybrid form contains 27 chromosomes.

BRYN MAWR COLLEGE,

July, 1907.

All of the figures are from camera drawings made with aid of Zeiss No. 12 compensating ocular and 2 mm. apochromatic objective. Some of the sketches were subsequently doubled in diameter by means of a drawing camera. These have been reduced one half in reproduction. The others are reproduced as drawn.

# THE SPERMATOGENESIS OF BUFO LENTIGINOSUS.

BY

HELEN DEAN KING, PH. D.

WITH 3 PLATES AND 2 DIAGRAMS IN THE TEXT.

Although the spermatocytes of amphibians seem to be exceptionally favorable material for a study of maturation phenomena, there has been considerable controversy among the workers on amphibian spermatogenesis regarding the character of the maturation divisions. Several investigators, among whom may be mentioned Meves, McGregor, and Eisen, maintain that both of the maturation divisions are longitudinal; Montgomery and vom Rath, on the other hand, claim that only one of the divisions is longitudinal, the other being a reduction division in the Weismannian sense. It seems necessary, therefore, that the behavior of the chromatin in the germ-cells of more species of amphibians should be studied in order that this point may be definitely determined.

With the exception of vom Rath (46, 47), Bühler (7), and Broman (6), the investigators who have recently studied the spermatogenesis of amphibians have worked on various species of the Urodela. Vom Rath's study of *Rana* was very fragmentary and his results have been severely criticised; the researches of Bühler on *Bufo vulgaris*, and of Broman on *Bombinator igneus*, were confined to the development of the spermatid into the spermatozoön. The spermatogenesis of the Anura is, therefore, practically an unworked field. The present paper records investigations on the common toad, *Bufo lentiginosus*, which form the starting point for a study of the chromatin relations in the germ-cells of various species of amphibians. It is only by a comparative study of many forms that one can safely draw conclusions for an entire class.

## MATERIAL AND METHOD.

Testes of adult toads killed at various times from April until September, and also testes of young toads with a body length of from 2 cm. to 6 cm. were used for these investigations. In both kinds of material like processes of development were found to be taking place, and it is evident that the spermatozoa formed anew each year in the adult undergo

a course of development similar to that of the spermatozoa which are first formed in the young.

The testes of the adult toad are cylindrical bodies lying directly in front of the kidneys. They measure from 10 mm. to 12 mm. in length and from 2 mm. to 3 mm. in width, the difference in size depending doubtless on the age of the toad. At the anterior end of each testis, and continuous with it, is a small, rounded structure, the so-called "Bidder's organ." This body is probably a rudimentary ovary, as several investigators have maintained, and it has seemingly nothing to do with the development of the spermatozoa.

Each testis is divided into a number of compartments, or follicles, separated by comparatively thick walls which contain numerous follicle cells. Each follicle is, in turn, divided into several smaller compartments, or cysts, which are separated from each other by much thinner walls. As a rule, all of the cells in a cyst are in approximately the same stage of development; but a single follicle may contain both spermatogonia and maturing spermatids. A transverse section of the testis, therefore, shows practically all stages in the development of the spermatozoa. As might be expected, testes of adult toads killed soon after the end of the breeding season contain large numbers of dividing spermatogonia; while the testes of toads killed in August or September contain relatively more spermatocytes and spermatids.

The structure of the testis of a young toad is similar to that of the adult except that there are fewer follicles and cysts. Until the toad has attained a body length of about 2 cm. the testes contain nothing but spermatogonia surrounded by follicle cells. After this time some of the cells evidently develop much faster than others, as spermatids can always be found among the spermatogonia and spermatocytes in the testes of toads 3 cm. long. When the young toad is about 5 cm. in length sections of the testes, except for their smaller size, cannot be distinguished from those of an adult killed in the early autumn.

Various fluids were used for fixation of the material, by far the most satisfactory being Flemming's solution (strong formula). Corrosive-acetic, which gives exceedingly good preparations of the egg, is but an indifferent fixing fluid for the testes as is also Gilson's mercurio-nitric solution. As a rule, the sections were stained with Heidenhain's iron-haematoxylin which was followed by erythrosin or orange G. Such a combination stain seems to differentiate the achromatic structures of the cell, particularly the attraction-sphere and the centrosome, as sharply as it does the chromatin. Safranin followed by lichtgrün and Her-

mann's gentian-violet and safranin also proved of value as they differentiate the chromatin very clearly; they cannot, however, be employed to advantage in a study of the cytoplasmic structures of the cell. Material fixed in corrosive-acetic (5 per cent acetic acid) was stained in all cases with Delafield's hæmatoxylin followed by orange G.

#### THE PRIMARY SPERMATOGONIA.

Testes of very young toads or those of adults killed in May and June contain many of the large, faintly staining cells to which La Valette St. George gave the name spermatogonia. In the testis of the adult these cells lie, almost invariably, close against the follicle membrane; they are rounded in outline and contain a very large polymorphic nucleus (Fig. 1). A nucleus of this type seems to be characteristic of the primary spermatogonia of amphibians, as it has been found in these cells by most of the workers on amphibian spermatogenesis. A number of small cells, similar in appearance to the cells of the follicle membrane, are always to be found flattened against the primary spermatogonia (Fig. 1, *F. C.*). La Valette St. George (52) was the first to describe such cells in the testes of amphibians; and more recently they have been found by Meves (36) in *Salamandra*, by Janssens (23) in *Triton*, and by Kingsbury (29) in *Desmognathus*. It seems very probable that the cells surrounding the primary spermatogonia have the same origin as those on the follicle membrane, and that they are concerned in some way with the formation of the cyst walls which appear as soon as the primary spermatogonia have divided into daughter-cells. The follicle cells are much less noticeable after the secondary spermatogonia are formed; they are crowded in the spaces between adjacent cysts and no longer surround the separate spermatogonia.

The resting nucleus of a primary spermatogonium contains an irregular linin meshwork on which are distributed minute, faintly staining granules of chromatin. A number of rounded nucleoli of various sizes are also scattered throughout the nucleus, being held, apparently, in the meshes of the nuclear reticulum. When material is examined that has been fixed in Flemming's solution and stained with any of the combination stains used, all of the nucleoli invariably take the chromatin stain. If, however, testes are fixed in corrosive-acetic and stained with Delafield's hæmatoxylin and orange G, some few of the nucleoli will stain with the orange, thus showing that they are plasmosomes; the greater number of the nucleoli, however, take the hæmatoxylin and are, therefore, to be considered as chromatin-nucleoli. Janssens also has

found two kinds of nucleoli in the primary spermatogonia of Triton; but I have not been able to confirm his statement that the plasmosomes pass out of the nucleus and serve as nourishment for the cytoplasm. In *Bufo* the plasmosomes are always found in the nucleus until the early prophase of mitosis, when they completely disappear, as do also the chromatin-nucleoli; the latter are doubtless used in the formation of the chromosomes.

In the resting stages of the primary spermatogonium, the centrosome is a minute, deeply staining, spherical body, lying in the cytoplasm near the nucleus and usually in one of the numerous indentations of the nuclear membrane (Fig. 1, *C*). The centrosome at this time is always inclosed in a rounded, granular attraction-sphere that is very similar to the one found by Rawitz (48) in the resting spermatogonia of *Salamandra*.

In addition to the centrosome and its attraction-sphere, there is present in the cytoplasm of the primary spermatogonium a round, or slightly oval, apparently homogeneous body that is considerably larger than the centrosome near which it is generally found (Fig. 1, *A*). Usually, though not invariably, this body is surrounded by a clear area which sharply marks it off from the cytoplasm. It is probable that this structure is to be identified with the "chromatoid Nebenkörper" found by Benda (1) in his study of mammalian spermatogenesis. Benda states that the chromatoid Nebenkörper has nothing to do with the centrosome or with the attraction-sphere, and that it is probably chromatin that has been ejected from the nucleus. He finds that this body disappears in the spermatid and he traces it back to the spermatocyte, but ventures no conjecture as to its probable function. In size and shape this body in the spermatogonia of *Bufo* greatly resembles the smaller chromatin-nucleoli; but in corrosive-acetic preparations stained with Delafield's hæmatoxylin and orange G, it invariably takes the plasma stain and appears as a condensed portion of the cytoplasm. I have not been able to determine the origin of this body which is present in the primary spermatogonia of the young toad long before metamorphosis; but, judging from its staining reactions, it is certainly not chromatin. As will be shown later, this body is undoubtedly concerned in the formation of the acrosome of the spermatozoön, and therefore I suggest for it the name acroblast as somewhat more appropriate than "chromatoid Nebenkörper."

When the spermatogonium is in the early prophase of mitosis, and before the nuclear membrane has broken down, the acroblast becomes constricted through the middle as shown in Fig. 22, *A*, and then divides. The

two acroblasts thus formed separate, and after mitosis is completed one of them is to be found in each of the daughter-cells. An equatorial section of the spindle of a primary spermatogonium during metakinesis occasionally shows the two acroblasts lying together in the cytoplasm near the chromosomes (Fig. 4, A); usually, however, the acroblasts separate before this time and are not to be seen in such a section of the cell. Not infrequently the acroblast can be found on the spindle among the chromosomes (Fig. 9, A). In such cases, if the preparation has been stained with iron-haematoxylin, the acroblast appears as a very small, round chromosome, and it might readily be mistaken for an "accessory" chromosome if its previous history had not been ascertained. The acroblast divides in each of the spermatogonial mitoses and also in both of the maturation divisions, so that one of these bodies is to be found in the cytoplasm of every spermatid. In the spermatid the acroblast undergoes a final division and one part migrates to the anterior end of the spermatid to form the acrosome; the other part remains in the posterior region of the spermatid and eventually disappears.

According to the researches of La Valette St. George (53), Meves (35), Benda (2), and McGregor (34), the primary spermatogonia in the amphibian testes divide amitotically and the descendants of these cells become functional spermatozoa. In *Salamandra*, according to Meves and Benda, this division takes place by means of the constrictive power of a ring-shaped centrosome; in *Amphiuma*, McGregor finds that the nucleus is divided by a simple cleft into two nearly equal parts. Although I have carefully searched through many sections of testes containing large numbers of primary spermatogonia, I have never been able to discover a single cell which I could be sure was dividing amitotically. In the large spermatogonia the nucleus is often found to be much more irregular in outline than that shown in Fig. 1, and not infrequently it appears to be composed of two or more large lobes which are connected only by a very narrow bridge of nuclear substance. In these cases, however, the outline of the cell is invariably rounded, and I have never found any indication of a division of the cytoplasm. If amitotic division of the primary spermatogonia occurs in the testes of *Bufo*, it is exceedingly rare and probably, as suggested by vom Rath (46), the cells dividing in this manner are in the process of degeneration and never develop into mature spermatozoa.

In every case in which I have observed the division of the primary spermatogonia the process has been taking place karyokinetically. In the early prophase of mitosis all of the nucleoli disappear and the

chromatin, which now stains very intensely, forms into an apparently continuous spireme which breaks into segments before the nuclear membrane disintegrates. There are twenty-four of these chromatin segments, this being the number that is characteristic of the somatic cells of the species. At first these segments are scattered throughout the nucleus, and the marked variation in their size can readily be seen. Figs. 2 and 3 show the total number of chromatin segments in a primary spermatogonium during the early prophase of mitosis; four of the segments are noticeably larger and longer than the rest; four others are very small; and the remaining sixteen segments are intermediate in size between these two extremes. One can readily arrange the chromosomes in twelve groups in which the two members of a group lie near each other and are approximately of the same size. Thus the "pairing" of homologous chromosomes in preparation for the maturation divisions already exists in the original parent cells, as Montgomery (39) has stated is the case in *Desmognathus* and *Plethodon*. A difference in the size of the chromosomes is found in the division stages of all generations of the secondary spermatogonia (Figs. 7-9), and also in both of the spermatocyte divisions (Figs. 43-45, 48-51, 56-57). This furnishes additional evidence in favor of the view advocated by Boveri, Montgomery, Sutton, and others that the chromosomes retain their individuality throughout all stages in the development of the germ-cells. After the nuclear membrane breaks down, the chromosomes condense into broad, V-shaped loops. Sections of the spindle during metakinesis (Figs. 4-5) show a marked difference in the size of the chromosomes; but at this time the chromosomes are so crowded together that it is impossible to determine whether they are arranged on the spindle fibres in any definite way.

About the time that the chromatin spireme breaks into segments preparatory to the division of the cell, the centrosome divides (Fig. 3) and a spindle forms, evidently of extranuclear material. The spindle fibres converge sharply at the poles where the minute centrosomes are clearly seen (Fig. 5). The attraction-sphere, which always surrounds the centrosome during the resting stages of the cell, disappears in the early prophase of mitosis, as it does in the spermatogonia of *Salamandra* (Meves, Rawitz), and of *Amphiuma* (McGregor). It is not again visible until the resting stages of the daughter-cells, as the centrosomes at the poles of the division spindle are totally devoid of any radiation.

## THE SECONDARY SPERMATOGONIA.

The secondary spermatogonia are much smaller than the parent cells, and, owing to compression, they are more or less polygonal in outline. The earlier generations of these cells have a conical shaped body with the apex of the cone turned towards the centre of the cyst in which they are contained. As the cells increase in number they are closely crowded together and the cell outlines become constantly more angular and irregular (Figs. 6-9). In the earlier generations of these cells the nucleus is polymorphic in form (Fig. 6), but in the later generations it is usually round or oval. In the resting cell the chromatin is again distributed on a linen meshwork in the form of small granules. These granules stain somewhat more deeply than during the earlier period and their distribution is not so regular (Fig. 6), as in many places groups of them become massed together so that the nuclear reticulum appears jagged and uneven. There are rarely more than five nucleoli in the nucleus of a secondary spermatogonium; of these one or two are plasmosomes, the others karyosomes.

I have never found the secondary spermatogonia dividing amitotically as they do occasionally in *Salamandra* according to Meves. The pro-phases of mitosis in these cells are essentially like those in the primary spermatogonia. All of the nucleoli disappear and the chromatin forms into a thick, apparently continuous spireme which breaks into twenty-four segments of different lengths (Fig. 7). The chromatin segments soon condense into V-shaped chromosomes which are somewhat smaller than those found in the primary spermatogonia.

The spindle in the secondary spermatogonia (Figs. 8, 9) is shorter and somewhat broader than that found in the parent cells (Fig. 5). It also has no radiation around the centrosomes at the spindle poles. The centrosome, attraction-sphere and the acroblast appear in the resting stages of the secondary spermatogonia as they do in the primary spermatogonia.

## THE SPERMATOCYTES.

- The last division of the secondary spermatogonia gives rise to a new generation of cells, the primary spermatocytes, in which occurs the interesting, yet perplexing, series of changes that brings about a reduction in the number of the chromosomes preparatory to the maturation mitoses.

The resting nucleus of the young spermatocyte usually occupies an eccentric position in the cell. It is invariably round or oval with a smooth contour (Figs. 10, 11) and never has the irregular outline com-

monly found in the cells of the preceding generations. In the very young spermatocyte the chromosomes are still distinct, and they appear as very short, thick rods which are connected by fine linin fibres (Fig. 10). In a slightly older cell, (Fig. 11), the chromosomes are found to be irregular in shape while the linin fibres connecting them are somewhat coarser and stain more intensely than in the younger cell. As the spermatocyte increases in size, the chromosomes gradually disappear, their substance going to form the nuclear reticulum which is constantly growing in amount (Figs. 12-14). At the stage of Fig. 13, minute granules of chromatin are first found scattered along the linin threads. As the substance of the chromosomes becomes distributed throughout the nucleus, the reticulum appears to be composed of a single series of deeply staining, rounded granules which lie so close together that it is almost impossible to make out the linin threads (Fig. 14).

At the end of the growth period of the primary spermatocyte (Fig. 15), the nucleus has increased enormously in volume, and it is very large in proportion to the size of the cell. At this time the chromatin is in the form of a granular, much convoluted spireme which is apparently continuous. The spireme always appears perfectly homogeneous and never shows any indications of a longitudinal splitting.

In the nucleus of the spermatocyte shown in Fig. 11, many of the chromosomes are found to be connected by two parallel linin threads. Such a pairing of the connecting fibres is much more noticeable in the later stages shown in Figs. 12-14. Judging from these figures alone, one might be inclined to think that each chromosome becomes split longitudinally during the formation of the nuclear reticulum in the young spermatocyte, and that the chromatin substance is later distributed along the linin fibres so that the paired threads found at the stages of Figs. 13 14, 18, 19, represent sister portions of longitudinally split chromosomes. This theory might indeed be plausible were the threads of the spireme invariably arranged in pairs at these stages in the development of the spermatocytes; but such is not the case. Sometimes, as shown at the top of Fig. 12, two chromosomes are connected by three linin threads and very often only one fibre connects two chromosomes. The pairing of the chromatin threads is most pronounced at the stage of Fig. 14, and it is rarely found at all at the end of the growth stage when the nucleus contains an apparently continuous spireme (Fig. 15). I do not, therefore, ascribe any special meaning to the pairing of the chromatin threads in the growth stages of the spermatocytes. The spireme is so long and so much convoluted at this time that it would

be very remarkable if some of the threads did not lie parallel for a longer or a shorter distance.

During the growth stages and until the formation of the first maturation spindle the centrosome is always to be found in the portion of the cell containing the greatest amount of cytoplasm (Fig. 15). As in the resting spermatogonia the centrosome is surrounded by a granular attraction-sphere which always appears homogeneous. The acroblast can be found in all of the resting stages of the spermatocytes (Fig. 14), lying usually near the centrosome (Fig. 15). It has the same appearance as in the earlier stages.

Soon after the primary spermatocyte has reached its maximum size, there begins a gradual condensation of the nuclear contents which can readily be followed from its beginning, shown in Fig. 16, to the stage shown in Fig. 22 where the entire contents of the nucleus forms a rounded mass in which it is not possible to make out any details of structure or to determine any of the changes that are taking place. McClung (33) has suggested that the term "synizesis" be applied to the "unilateral or central contraction of the chromatin in the nucleus during the prophase of the first spermatocyte." I shall, therefore, use this term with reference to the stage shown in Fig. 22, in order to avoid the confusion that has resulted from the misuse of the word "synapsis."

The first evidence of the beginning of synizesis is the appearance, near the centre of the nucleus, of a deeply staining mass of tangled chromatin threads from which long loops of the spireme go out in all directions (Fig. 16). At a slightly later stage (Fig. 17), this central mass appears much larger and there are correspondingly fewer loops of the original spireme to be seen. During these early stages in the process of condensation there is frequently found an apparent pairing of the chromatin loops as shown in Figs. 18 and 19; but, as in the growth stages of the spermatocytes, this pairing of the chromatin loops is by no means a constant phenomenon and I cannot but regard it as purely accidental. At the stage of Fig. 20, the greater part of the contents of the nucleus has become condensed into an apparently homogeneous mass from which only a few short filaments of chromatin project. These filaments are very much finer than those of the original spireme and most of them lie parallel with their free ends invariably pointed to that part of the cell in which the centrosome lies. Such a condition of the nucleus as that shown in Fig. 20, is met with so frequently that it is evidently a well defined stage in which there is a definite orientation of the nuclear contents. The gradual condensation of the contents of the

nucleus continues until all of the chromatin is collected in an oval or spherical mass lying usually close to the nuclear membrane with which it is connected by very fine fibres (Figs. 21, 22). In preparations stained with iron-haematoxylin in the usual manner this mass of chromatin appears perfectly black and apparently homogeneous (Fig. 22); if, however, the greater part of the stain is extracted from the sections, this structure is found to be composed of a tangled mass of very fine, granular filaments among which are a number of rounded, deeply staining nucleoli (Fig. 23). These nucleoli, judging from their staining reactions, are not plasmosomes, but more condensed portions of the chromatin substance which retain the haematoxylin with great tenacity.

It can readily be demonstrated that this condensation of the nuclear contents is an actual constructive stage in the normal course of the development of the spermatocytes of *Bufo* and is not "the expression of a running out in the spermatogonium stock and represents a tendency towards degeneration," as claimed by Kingsbury (29) for *Desmognathus*. In the testes of adult toads killed at any time during the summer months, large numbers of spermatocytes can be found showing every step in this process from the stage shown in Fig. 16 to that of Fig. 22, no matter what method of fixation or of staining has been employed. In the testes of toads killed in September and October, the maturation divisions are rarely found, and most of the cysts are filled with spermatids and maturing spermatozoa. If the spermatocytes in which a condensation of the nuclear contents occurs during the summer months are in a state of degeneration, one would expect to find the testes of toads killed in the autumn filled with a large number of cells in the process of disintegration; but such is not the case. It seems quite improbable that large numbers of cells could disintegrate and the débris be disposed of so quickly that it is impossible to obtain any late stages in the process.

I have never found a condensation of the chromatin in the spermatogonia as Kingsbury has described for *Desmognathus*, and I am unable to confirm his statement that "contraction figures do not occur constantly in spermatocytes." In *Bufo* the synizesis stage is most frequently met with in the testes of toads killed in the early summer months simply because at this time the testes contain relatively more cells in this particular stage of development. Contraction figures are, however, to be found in practically any section of the testis, no matter what time of year the toad is killed, and they are found in relatively the same abundance in the testes of young toads as they are in the adult.

From a study of the spermatogenesis of *Batrachoseps attenuatus* Janssens (24) concludes that a condensation of the nuclear contents occurs only when material is badly preserved, and he states that cells of this type are commonly met with in the central mass of tissue where the reagents have not penetrated sufficiently well. Such an explanation will not account for the presence of synizesis in the spermatocytes of *Bufo*. Cells in this stage of development are found as frequently in the cysts at the periphery of the testis as in those at the centre, and not infrequently a cyst filled with spermatocytes in the synizesis stage lies adjacent to a cyst containing dividing cells in which the chromosomes, centrosomes, and achromatic structures are remarkably clear and well preserved. I do not think it possible that a method of fixation that would cause the nuclear contents of one set of cells to become as contracted as that shown in Fig. 22, would not also distort the contents of the other cells of the surrounding tissue to a greater or a less degree.

In none of the amphibians that have so far been studied is the condensation of the chromatin in the primary spermatocytes as complete as in the case of *Bufo*. Janssens (23) states that in the resting stages of the spermatocytes of *Triton* all of the substance of the nucleus is collected in a mass from which numerous filaments extend out to various parts of the nuclear membrane. The synizesis stage in *Triton* is, according to Janssens, about like the stage of condensation in *Bufo* shown in Fig. 20. In *Salamandra*, *Amphiuma*, *Batrachoseps*, and *Plethodon*, according to the investigators who have worked on these amphibians, there is no stage in the development of the spermatocytes in which a condensation of the nuclear contents occurs. The spireme formed after the last spermatogonial division splits longitudinally at a stage about like that of my Fig. 15, and it subsequently breaks into the reduced number of chromosomes. If a condensation of the nuclear contents of the spermatocytes is not characteristic of these cells in all amphibians, it certainly is not confined to *Bufo* and *Triton* as I have already found it in several species of *Rana* and in two of the *Urodela*.

It is obviously impossible to determine what changes are taking place in the chromatin during the time that the substance of the nucleus is massed together in the synizesis stage, as no definite structures, other than those shown in Fig. 23, can be made out even in the most favorable preparations. Judging from the results that have been obtained from a study of the spermatocytes in other species in which a condensation of the chromatin does not occur, it is probable that during synizesis the chromosomes become united end to end in a spireme which later seg-

ments into the reduced number of chromosomes. The "synapsis" stage, to use the term introduced by Moore (41) to designate the stage in which the pseudo-reduction in the number of chromosomes occurs, is in *Bufo* co-incident with the synizesis stage as it is in *Triton*, *elasmobranchs*, and various other forms.

A section through a spermatocyte as the cell is emerging from the synizesis stage is shown in Fig. 24. Against one side of the nucleus is a dense irregular mass of chromatin from which extend the loops of an apparently continuous spireme. This spireme differs from that found in the spermatocytes at the end of the growth period, in that it is shorter, thicker, and instead of being granular is very "mossy" in appearance. A slightly later stage in which the mass of chromatin against the nuclear membrane has still further decreased is shown in Fig. 25. At the end of the synizesis stage the nucleus contains a deeply staining, apparently continuous spireme (Fig. 26) which has a much smoother outline than the spiremes shown in Figs. 24 and 25. I have never found any indications of a longitudinal splitting of the spireme that is so characteristic of the post-synapsis stages in the spermatocytes of the *Urodela*.

Soon after the stage shown in Fig. 26, the spireme shortens somewhat and, consequently, becomes considerably thicker than before (Fig. 27). Subsequently certain portions of the spireme stain much more lightly than the rest, these lighter staining regions marking the location of the transverse divisions by which the spireme is later broken into the reduced number of chromatin segments (Fig. 28). The next step in the process of separating the chromosomes is seen in Fig. 29, where the spireme is found to be constricted at various places. In many cases, before this first constriction of the spireme is completed, a second constriction appears through the middle of each segment so that the chromosomes that are finally produced from the spireme have the shape of dumbbells (Fig. 30). It is very probable that the constriction in each segment marks the line of union of the two chromosomes that were united end to end in synapsis; and, as will be shown later, this constriction also marks the location and the direction of the division of the chromosomes in the first maturation mitosis of the spermatocytes. When the chromosomes have the shape of dumbbells at the stage of Fig. 30, they undergo no further changes in form until the anaphase of the first maturation mitosis. There is no indication of the second division in this type of chromosome until the late anaphase when the chromosomes are again constricted into dumbbells (Fig. 51). If one traces the various steps in the development of the chromosomes from the stage represented by Fig. 27 until

the time that mitosis is completed by the division of the chromosomes through the middle of the dumbbells (Figs. 41, 42, 44, 45, 48), there seems no possibility of avoiding the conclusion that the first maturation mitosis in the spermatocytes of *Bufo* is a transverse or reduction division in the Weismannian sense, separating chromosomes that were united end to end in synapsis.

In some of the spermatocytes the spireme formed after the synizesis stage does not produce dumbbell-shaped chromosomes, but breaks into rectangular blocks (Fig. 35). These rectangular shaped chromosomes also maintain their definite shape up to the metaphase, and are never divided either for the first or for the second maturation mitosis before this time. Owing to this persistence in the shape of the chromosomes there can be no doubt but that here too the first division is a reduction division; as in the anaphase the chromosomes are nearly square and not long and narrow as would be the case if they were divided longitudinally (Fig. 52).

An examination of Figs. 30-36 will show that before the separation of the chromosomes there is always a considerable variation in the size of the segments in the same nucleus. This difference in the size of the chromosomes is maintained throughout all of the subsequent stages of mitosis and shows very clearly in the equatorial plate of the first maturation spindle shown in Fig. 43. All twelve of the chromosomes are present in this case and two of them are considerably larger than the others. In the early prophase of the division of the primary spermatogonium shown in Figs. 2 and 3, there are four of the chromosomes that are noticeably larger than the rest. It seems very probable that these chromosomes maintain their individuality throughout all of the spermatogonial divisions and are united two and two in synapsis to reappear in the prophase of the first maturation mitosis as the two bivalent chromosomes that are readily distinguished from the others by their size. If this is true for four of the spermatogonial chromosomes it is, of course, true for all. Thus the twelve chromosomes shown in Fig. 43 are bivalent structures formed by an end to end union of homologous chromosomes which, as Montgomery (39) has shown, are to be traced back through the divisions of the secondary spermatogonia to the primary spermatogonia.

The difference in the size of the chromosomes on the first maturation spindle is strikingly illustrated in Fig. 48 where one of the largest of the chromosomes lies next to one of the smallest, the mass of the former being more than twice that of the latter. In the metaphase and early anaphase of the second maturation division (Figs. 56, 57), one can also

perceive a difference in the size of the chromosomes, although this difference is not so marked as in the first mitosis because of the smaller size of the chromosomes. Many investigators have already noticed a variation in the size of the chromosomes in the maturation divisions of the germ-cells. Were the reasons for this phenomenon known, they would doubtless aid materially in solving many of the vexing problems of heredity.

In a great majority of the spermatocytes the post-synapsis stages of development do not follow the well marked types described above, but are irregular and somewhat difficult of analysis. In many cases the spireme, instead of breaking into dumbbell or rectangular-shaped segments, forms the ring-shaped chromosomes that are so characteristic of the prophases of the first maturation mitosis in the spermatocytes of other animals. In most of the cases that have been described, the ring-shaped chromosome is produced by the longitudinal splitting of a chromatin segment which opens through the middle region and remains united at the ends, a typical case being that of *Gryllotalpa* as described by vom Rath (45). In *Bufo* the method of ring formation is somewhat peculiar. The spireme formed after the synzesis stage constricts into oval segments (Figs. 32-34), and usually, before the constriction is completed, the segments open through the middle, thus forming typical ring-shaped chromosomes which may remain connected for some time (Figs. 31, 32, 33, 36). In *Bufo*, therefore, the opening in the ring is not the space between univalent chromosomes, as maintained by Montgomery (38) for *Desmognathus* and *Plethodon*, but represents the longitudinal splitting of a bivalent chromosome that is completed in the second maturation mitosis. The first maturation mitosis, which is always seen at this stage in the dumbbell-shaped chromosomes (Figs. 30, 37, 38), is not, as a rule, visible in the ring-shaped chromosomes until they have entirely separated and condensed into tetrad groups, as do the ring-shaped chromosomes in *Gryllotalpa* (Fig. 39). Before the first maturation spindle is formed the ring-shaped chromosomes become scattered throughout the nucleus (Fig. 40). They are at this time usually round or oval in form, but occasionally they are distinctly diamond shape (Figs. 32, 40). In only two cases have I ever seen any indication of a division of the ring-shaped chromosomes preparatory to the first maturation mitosis. One of these cases is shown in Fig. 32, where, at the four corners of the diamond, dark lines divide the chromosome into four parts, thus plainly indicating that the chromosome has already divided for both of the maturation mitoses.

It is apparently a matter of little, if of any, importance which of

these various forms the chromosomes assume in the prophases of the first maturation mitosis, as frequently in the same nucleus some of the chromosomes have the form of dumbbells while others are in the shape of rings (Fig. 38). Nuclei containing chromosomes of different shapes are perhaps the most valuable for a study of this period in the development of the spermatocytes, as they show very clearly how the different types are related to each other and to the original spireme. Fig. 38 shows a section of a spermatocyte in which the chromosomes are of mixed types. Three dumbbell-shaped chromosomes, in which the direction of the first maturation mitosis is indicated by the constriction in the middle of the chromosomes, are connected with a ring-shaped chromosome which shows only the second division. As these chromosomes are still united end to end by linin fibres there can be no question of the relation of these two types of chromosomes to each other and to the original spireme.

A section of another spermatocyte of the same character is shown in Fig. 39. In this case not only are the chromosomes of different types but they are also in slightly different stages of development. As the chromosomes are entirely separated it is not possible to determine their relation to the original spireme. This cell is unique in that it shows in the same nucleus chromosomes in which the first or the transverse division has already begun (dumbbells), chromosomes in which the second or the longitudinal division only is apparent (rings), and one chromosome which has already divided for both of the maturation mitoses (tetrad).

Although ring-shaped chromosomes are found abundantly in the pro-phases of the first maturation mitosis, I have never found one of them in the equatorial plate of the spindle where they are always to be found in *Salamandra*, *Amphiuma*, and others of the *Urodela*. In a study of the spermatogenesis of *Pedicellina americana*, Dublin (11) found that the chromosomes have the form of rings in the prophase of the first maturation mitosis. When the spindle is formed these chromosomes elongate considerably, but still retain their ring-shaped character during the early metaphase. In the late metaphase the chromosomes lose their former shape and condense into elongated rods which divide transversely, the division being reductional. Thus *Pedicellina* seems to bridge over the gap between such forms as *Salamandra* and *Amphiuma* in which the ring-shaped chromosomes persist throughout the entire metaphase of the first maturation mitosis, the ensuing division being of the hetero-

typic type, and forms like *Gryllotalpa* and *Bufo* in which rings give place to tetrad groups before the metaphase and division is reductional.

Where tetrads are formed in the prophases of the first maturation mitosis it is, of course, not possible to determine whether the first or the second division is reductional. As the first maturation division of the chromosomes that have the shape of dumbbells and also of those that take the form of rectangular blocks is transverse, it seems safe to assume that the tetrads are so placed on the spindle that univalent chromosomes are separated in the first mitosis.

The formation of the first maturation spindle evidently takes place very quickly, as I have been able to find only a comparatively few stages in its development. Soon after the synizesis stage the centrosome divides and the attraction-sphere disappears as it does before the formation of the spindle in the spermatogonial mitoses. As the centrosomes move apart, each becomes the centre of a very small aster formed, presumably, of the substance of the attraction-sphere (Figs. 36, 41). As a rule, the centrosomes lie at this time very close to the nuclear membrane which is somewhat irregular in outline. After the disappearance of the nuclear membrane, a small spindle is found with the centrosomes, surrounded by small asters, at the spindle poles (Fig. 42). The spindle grows rapidly, probably at the expense of nuclear material, and when fully formed in very large proportion to the size of the cell (Figs. 44-53). After the stage of Fig. 42 every trace of the polar radiation disappears and the centrosomes are totally devoid of any radiation in all subsequent stages.

During, or soon after the synizesis stage, the acroblast becomes dumb-bell shape (Fig. 22, *A*) and then constricts into two rounded bodies (Fig. 26). This division takes place before the centrosome divides, for, as shown in Fig. 26, one can occasionally find a section of a cell containing the centrosome surrounded by its granular attraction-sphere and two acroblasts. During the formation of the spindle the acroblasts usually separate and in the metaphase are to be found some distance apart (Fig. 48). Occasionally, as in the spermatogonial divisions, the acroblasts lie close together in the equatorial region of the spindle and appear as very small chromosomes (Fig. 45, *A*). During the anaphase the acroblasts have apparently no definite position in the cell as they may be found on the spindle, near to it, or close against the cell wall (Figs. 50, 53). They are, however, at this time always on opposite sides of the equator of the spindle, and when cell division takes place one acroblast goes to each of the daughter-cells.

During the late anaphase of the first maturation mitosis, the chromosomes become greatly crowded together and appear to fuse into an amorphous mass in which the outlines of the separate chromosomes are completely lost (Fig. 53). Whether there is a true fusion of the chromosomes at this time I have been unable to determine. Kingsbury has found a similar massing of the chromosomes at the poles of the first maturation spindle in the spermatocytes of *Desmognathus*, and he believes that the chromosomes do not lose their identity at this time although they become inclosed in a nuclear membrane. Division does not take place simultaneously in all of the spermatocytes of a cyst; sometimes only a single cell will be found dividing while all of the other cells of the cyst are in the early or late prophase of mitosis.

As in *Salamandra* and *Triton*, there is no resting nucleus formed between the two maturation divisions. Sometimes the centrosomes divide for the second mitosis during the early anaphase of the first mitosis (Fig. 50), but as a usual thing division does not take place until much later. As in the prophase of the former divisions, each centrosome is, for a time, the centre of a small aster (Fig. 54). As the spindle for the second mitosis forms, the asters disappear and in the fully formed spindle the fibres converge sharply to minute centrosomes which show no traces of a radiation.

During the formation of the second maturation spindle, the chromosomes remain massed together, as shown in Fig. 54, and the changes taking place in them cannot be determined. In rare instances this amorphous mass of chromatin may be found at the equator of the fully formed spindle (Fig. 55), but as a rule the chromosomes are separated at this time. Part of an equatorial plate of the second maturation spindle is shown in Fig. 56. Nine dumbbell-shaped chromosomes are seen which, as one might expect, are very much smaller than those found on the first spindle. The fact that at this stage also the chromosomes are of different sizes seems to indicate that the chromosomes maintain their individuality during the period between the two maturation divisions when they are apparently fused into an irregular mass. The second division must take place much more quickly than the first, as I have not been able to find more than two or three dividing cells in sections of the testis containing hundreds of cells in earlier or slightly later stages of development. In all of the spindles that I have seen, the chromosomes were invariably in the form of dumbbells in the metaphase (Fig. 56). In the anaphase, each dumbbell is separated into two nearly spherical portions (Fig. 57) which are inclosed in a membrane

before the division of the cell is completed. The second mitosis is longitudinal, completing the division that is begun in some of the chromosomes at the stage of Fig. 31, and in others not until the late anaphase of the first mitosis (Fig. 51).

#### THE SPERMATIDS AND SPERMATOZOA.

The nucleus of the young spermatid occupies a very eccentric position, lying in the portion of the cell that is to become the head of the spermatozoön (Fig. 58). At first the chromatin is distributed throughout the nucleus in the form of angular shaped blocks connected by fine linin threads (Fig. 58); later it is spread out in an irregular reticulum (Figs. 59-65). During the development of the spermatid the nucleus loses its rounded shape and becomes greatly elongated, finally appearing in the mature spermatozoön as a cylindrical, perfectly homogeneous body that has great affinity for all chromatin stains.

The centrosome is always found in the part of the cell containing the greatest amount of cytoplasm. It is not surrounded by a granular attraction-sphere as in the resting stages of the preceding generations, but is inclosed in a clear, round or oval vesicle which is sharply marked off from the surrounding cytoplasm (Figs. 58, 59, etc.). Whether the substance of this vesicle is derived from the attraction-sphere of the spermatocytes I have not been able to determine. The attraction-sphere disappears in the prophase of the first maturation mitosis and I have found no traces of it in later stages, unless, indeed, the radiation found for a short time around the centrosomes as the second maturation spindle is forming is derived from the substance of the attraction-sphere.

In the early stages of the development of the spermatids, the vesicle inclosing the centrosome may lie freely in the cytoplasm (Fig. 60), or it may be in contact with the nuclear membrane (Fig. 58); in the latter case it marks the region of the nucleus that is to become the posterior end of the sperm-head, as the vesicle itself is the anlage of the middle-piece of the spermatozoön. The centrosome divides soon after the stage shown in Fig. 58, and in favorable preparations one can see that the two centrosomes are connected by a very fine, thread-like fibre (Figs. 59, 60). As the centrosomes move apart, the vesicle inclosing them elongates and later it becomes much flattened where it presses against the nuclear membrane (Figs. 61, 62, etc.). The middle-piece anlage appears perfectly transparent during all stages of its development and shows not the slightest affinity for either hæmatoxylin or safranin stain. In the mature spermatozoön, the middle-piece has the same diameter as

the sperm-head and forms a well marked division between the head and the tail (Fig. 71, *M. P.*).

One centrosome, which for convenience I shall call the "inner" centrosome, moves to the edge of the vesicle in contact with the nucleus and for a time appears as a small rounded projection from the middle of the posterior nuclear wall (Figs. 62, 63, 65). Later this centrosome becomes imbedded in the deeply staining substance of the sperm-head; whether it aids in the formation of the middle-piece, as Meves finds is the case in the spermatids of *Salamandra*, I have been unable to determine. The "outer" centrosome, still keeping its connection with the inner centrosome, moves to the posterior end of the vesicle and from it a fibre grows out that later forms the axial-filament of the tail (Figs. 61-63, etc.). In the spermatids of *Salamandra* and of *Amphiuma*, the outer centrosome becomes ring-shaped and later divides into two parts: one part remains at the posterior end of the middle-piece; the other part migrates down the axial-filament to the beginning of the end-piece of the tail. Owing to the fact that the spermatids in the testis of *Bufo* are very much smaller than those of the *Urodela* and that the posterior region of the cell shows little affinity for either plasma or chromatin stains, it is very difficult to follow the history of the outer centrosome. As far as I have been able to determine, this centrosome never divides, but remains at the posterior end of the middle-piece where it soon becomes disc shaped (Figs. 66-68), and later flattens considerably (Figs. 69-70). In favorable preparations of the mature spermatozoön, this centrosome appears as a more deeply staining mid-portion of the posterior border of the middle-piece; in many cases it cannot be found at all.

In the young spermatid, the acroblast appears round or oval and homogeneous as in the earlier stages. It is always found in the posterior region of the cell, usually lying some distance from the centrosome from which it is readily distinguished on account of its larger size (Fig. 58). During an early period in the development of the spermatid, the acroblast again divides, and occasionally a cell is found containing both of the centrosomes and two acroblasts which are considerably smaller than those found in the spermatocytes (Fig. 63). One of the acroblasts remains in the posterior part of the cell (Figs. 66, 68), and as the tail forms it stains less intensely than before and subsequently disappears. The other acroblast moves gradually towards the anterior end of the spermatid, as shown in Figs. 65-67. Transverse sections of the spermatids will occasionally show this acroblast lying just outside of the

nucleus surrounded, as in the earlier stages, by a clear area (Fig. 64). The acroblast finally reaches the anterior end of the cell (Fig. 67), and then comes in contact with the nuclear membrane (Fig. 68). Subsequently the acroblast flattens against the apex of the nucleus (Fig. 69) and seems to fuse with it, forming a deeply staining, cap-like body (Fig. 70, 71, *Ac.*). The cytoplasm in the anterior region of the cell later forms an awl-shaped apical body in front of the acrosome, which stains very faintly with plasma stains (Fig. 71).

During the later growth stages of the spermatids, the cysts inclosing them become disorganized and a cavity appears in the middle of the follicle. This cavity contains a considerable amount of *débris* formed from the degeneration of the cyst membranes and the follicle cells. Some of this *débris* is in the form of large and small granules which take the chromatin stain. It is, therefore, somewhat difficult to follow the history of the acroblasts, as frequently a spermatid will be found that appears to contain several rounded, deeply staining granules of about the same size as the acroblast. Fortunately, the acroblast in the spermatid is almost invariably surrounded by a clear area both before and after it has divided (Figs. 58, 61, etc.), and thus it can usually be distinguished from granules of *débris* that often appear to be a part of the cell. In studying the history of the acroblast I have made use of sections of the testis that were purposely crushed and broken; as by such means the spermatids, which are normally crowded close together, are separated and partially freed from other material in the follicle.

The mature spermatozoön of *Bufo lentiginosus* has already been described and illustrated (King, 26). The head is a long cylindrical structure which is seemingly homogeneous after the usual staining with iron-hæmatoxylin. If, however, the stain is partially extracted, as was done in the preparation from which Fig. 71 was drawn, the head appears grayish in color and there are two deeply staining regions, one at the anterior, the other at the posterior end. It is evident that the parts of the sperm-head that retain the stain with the greatest tenacity mark the location of the two bodies that entered the nucleus at an early period in the development of the spermatid. At the posterior end of the sperm-head lies the inner centrosome which entered the nucleus of the spermatid just before the stage of Fig. 66; and at the anterior end is the acroblast which fused with the nucleus at about the stage of Fig. 69. Both of these structures evidently persist in the mature spermatozoön and retain their great affinity for the iron-hæmatoxylin stain.

The middle-piece of the mature spermatozoön (Fig. 71, *M. P.*) has

the same diameter as the sperm-head, but it is sharply marked off from it and shows not the slightest affinity for chromatin stains. In rare instances, as shown in Fig. 71, a slender fibre extends through the entire middle-piece. This fibre is evidently the connection between the centrosome at the posterior end of the middle-piece and the centrosome imbedded in the end of the sperm-head which has persisted through all stages in the development of the spermatid. As a rule, the connection between the centrosomes is broken after the stage of Fig. 65, and in the older spermatids and in the spermatozoa the middle-piece appears perfectly homogeneous.

The tail of the spermatozoön is very long and it is composed of two filaments which are connected by a thin, transparent, undulating membrane. The axial-filament, which grows out of the outer centrosome, is somewhat thicker than the marginal-filament and it extends some distance beyond the latter to form the end-piece of the tail.

#### GENERAL DISCUSSION.

As long ago as 1887, Flemming (15) described typical tetrad groups in the testes of *Salamandra*, although he considered these structures to be "anomalies" and not normal stages in the development of the spermatozoa. Later vom Rath (46, 47) maintained that tetrads are normally present in the testes of *Rana* as well as of *Salamandra*, and that one of the maturation divisions is a transverse or reduction division in the Weismannian sense. With but few exceptions, all of the investigators who have recently worked on the spermatogenesis of amphibians have insisted that both of the maturation divisions are equatorial, and that normally tetrad groups are not present in the spermatocytes. This later work on amphibians, and the seemingly exhaustive studies of Brauer (5), Boveri (3), and Hertwig (22) on the germ-cells of *Ascaris*, together with the work of Strasburger (56, 57) and other botanists on the flowering plants, has given strong support to the view that reduction in the germ-cells can be effected either by a double longitudinal division or by one transverse and one longitudinal division, the division of the chromatin substance being the main thing and the manner of its achievement quite secondary. The most recent studies on spermatogenesis, and ovogenesis, and experiments made by Boveri, Morgan, and others, have seemed to show that the chromosomes maintain their individuality from one generation to the next. If this is true, then in the maturation mitoses, reduction must in all cases be brought about by one transverse

and one longitudinal division of the chromosomes, as the individuality of the chromosomes is lost if two equational or two transverse divisions occur.

Sebaschnikoff's (5) re-examination of the ovogenesis of *Ascaris* has made it doubtful whether, after all, the tetrad groups are here formed by a double longitudinal division; Boveri (4) and Montgomery (40) have more recently brought forward strong arguments in favor of the occurrence of a reduction division in the germ-cells of *Ascaris*; and Strasburger (58), after a re-examination of his material, is now of the opinion that a reduction division also occurs in the higher plants. It remains only to bring the investigations on the germ-cells of the amphibians into line with these later investigations on other forms to establish the rule that there is one reduction and one equational division in the maturation of the germ-cells of all animals and plants so far investigated. There is, apparently, a great diversity in the way in which reduction is accomplished in the various forms.

Most of the investigators who have worked on the spermatogenesis of the Urodela agree that, in the early prophase of the first maturation division, the reduced number of chromosomes appears in the form of U- or V-shaped loops; and they have tacitly assumed, if not expressly stated, that each loop is a bivalent structure being composed of two chromosomes united end to end in synapsis. Later these chromatin loops split longitudinally and the sister-portions of each loop remain united at the ends, opening up through the middle to form ring-shaped chromosomes. In metaphase the rings are said to be placed on the spindle in such a way that the plane of the union of the two halves of the chromosomes lies in the equator of the spindle. The heterotypic division which follows separates sister-portions of the longitudinally split chromosomes and is therefore an equational division. In the anaphase the V-shaped chromosomes again split longitudinally preparatory to the second maturation mitosis which is also an equational division.

Montgomery, who is a firm advocate of the view that the first maturation mitosis must necessarily be reductional, has investigated the prophase of the first maturation division in the spermatocytes of two amphibians, *Plethodon* and *Desmognathus*, and his interpretation of the ring-formation in these forms differs considerably from that given by Meves, McGregor, and Eisen (11). In the early prophase of mitosis, Montgomery also finds the reduced number of chromosomes in the form of loops which he considers to be composed of two chromosomes united end to end in synapsis; each arm of a loop representing one chromo-

some and the angle of the loop indicating the place of their union. At a later stage the free ends of each loop become cemented together forming ring-shaped chromosomes. "Hence in the typical chromosome of the ring form, the space enclosed by the chromosome is the space between two univalent chromosomes and has nothing to do with the longitudinal split." Montgomery asserts that the longitudinal splitting of the chromosomes in preparation for the second maturation mitosis appears in the arms of the loops before the rings are formed, and that it has been overlooked by the other investigators, as it is completely hidden in the metaphase and only reappears in the anaphase as a longitudinal splitting of the daughter chromosomes. Montgomery believes, therefore, that the so-called heterotypic division in the species of amphibians that have so far been investigated is a true reduction division in the Weismannian sense and that the second division only is a longitudinal one. He ventures the prediction that this will be found true for all forms in which the heterotypic division is found to occur.

The interpretation given to the ring-formation by Montgomery has been criticised by Janssens and Dumez (25) who insist that in *Plethodon* both of the maturation divisions are longitudinal. Janssens (24), however, in a more recent paper dealing with the development of the spermatocytes of *Batrachoseps attenuatus*, seems to have changed his former view as he states that "il est extrêmement probable, pour ne pas dire plus, que les 12 anses du bouquet chez le *Batrachoseps* résultent de la soudure deux à deux suivant toute leur longueur des 24 chromosomes des dernières cinèses somatiques." This conjugation of the chromosomes leads later to the formation of dyads "qui seront séparées pendant la première cinèse de maturation ou hétérotypie. Il est donc très probable que les deux spermatocytes II reçoivent chacun 12 chromosomes entiers, c'est-à-dire la moitié des 24 chromosomes des dernières cinèses spermatogoniales." Besides supporting Montgomery's contention that the first maturation division in the spermatocytes of amphibians is a reduction division, this work of Janssens is very valuable in another way, as it is the first work on the spermatogenesis of amphibians that suggests the possibility of a side by side union of the chromosomes during synapsis. I do not see why it is necessary to assume, as have the majority of the investigators on amphibian spermatogenesis, that the chromosomes must always unite end to end in synapsis. Evidence is not lacking that a conjugation of the chromosomes in pairs may take place previous to the first maturation division. Thus Rückert's (49,50) early observations on the oögenesis of *Pristiurus* seems to show a side by side union of the

chromosomes, although Rückert considers that the paired arrangement of the chromosomes may be due to "eine eigentümliche Langsspaltung der Chromosomen im Dyaster der letzten Teilung des Ureies." More conclusive evidence is given by Calkin's (8) work on *Lumbricus*. In this form the spireme first splits longitudinally and then segments into the somatic number of segments, 32. These double segments then conjugate in pairs forming 16 tetrad groups. The first maturation division is a reduction division, separating the pairs of chromosomes that conjugated to form the tetrads. More recently Steven's (55) investigations on the germ-cells of Aphids have shown that there is in these forms undoubtedly a pairing of the chromosomes previous to the first maturation division. If a more extended investigation of the spermatocytes of the Urodela should make it seem probable that Janssen's interpretation of synapsis in *Batrachoseps* can be extended to other species, then univalent chromosomes will be separated in the first mitosis in all such cases, and the maturation divisions in this group of amphibians will readily fall in line with those of all other accurately known forms.

In studying the spermatogenesis of *Bufo* I have been fortunate enough to find practically all stages in the development of the primary spermatocytes from the time that the cell is formed (Fig. 10) until the completion of the first maturation division (Fig. 53); and I have traced, as carefully as possible, the complex changes taking place in the chromatin at this time. In the young spermatocyte shown in Fig. 10, the chromosomes are distinct and connected by fine linin fibres. At this stage the cells are very small and many of the chromosomes are crowded against the nuclear wall so that I have not been able to make out their number satisfactorily. As there are certainly many more than 12 chromosomes in the nucleus at this time, I am very sure that pseudo-reduction has not yet taken place and that the somatic number of chromosomes, (24) is present. As shown in Fig. 10, the chromosomes in many cases appear to be connected end to end, especially is this noticeable in the chromosomes that lie against the nuclear wall. In the later development of the spermatocytes the chromatin substance becomes distributed on the nuclear reticulum and all traces of the individual chromosomes is lost. If, however, an end to end union of the chromosomes is established at or before the stage of Fig. 10, it is safe to assume that this connection is not broken during the later growth stages of the spermatocytes.

I have never found any evidence of the longitudinal splitting of the spireme in the young spermatocytes that has been found at the same

stage of development in the spermatocytes of several of the Urodela. Up to the stage of Fig. 14, the chromatin reticulum in the spermatocytes of *Bufo* is very fine and one can readily see that the chromatin granules are arranged serially. The continuous spireme found at the stage of Fig. 15 is always perfectly homogeneous. Although I have examined large numbers of spermatocytes very carefully with this particular point in mind, I have never been able to find a single cell in which there was the slightest evidence of a longitudinal splitting of the spireme at the stage of Fig. 15, nor during any stages showing the condensation of the nuclear contents. I have already stated that I regard the apparent pairing of the chromatin threads shown in Fig. 13, 14, 18, 19, as purely accidental. In most cases the parallel threads are some distance apart, this would probably not be the case if a longitudinal splitting of the spireme had occurred; again, the threads are never connected by fine fibres, as is usually the cases with sister portions of a longitudinally split chromatin skein; most important of all, during the early stages of the formation of the nuclear reticulum, the threads are always composed of a single series of chromatin granules and they are all of the same thickness whether they are single or in pairs.

Recently several investigators, among whom may be mentioned A. and K. E. Schreiner (54), and von Winiwarter (61), have maintained that the apparent longitudinal splitting of the chromatin loops during or soon after the "bouquet" stage is, in reality, a folding together of the chromatin filaments so that two of them come to lie parallel and thus produce the appearance of a split filament. Synapsis, according to this interpretation, is brought about by a side by side conjugation of the chromosomes and not by an end to end union. A folding together of the parts of the chromatin skein is exactly what occurs in the young spermatocytes of *Bufo* at the stages of Figs. 13, 14, 18, 19; but this arrangement always occurs before the synizesis stage and never after it, and it is found in only a small minority of the spermatocytes. Were this pairing of the chromatin threads interpreted as a conjugation of univalent chromosomes that is to persist throughout the synizesis stage and until the metaphase of the first maturation division, then, as will be shown later, both of the maturation divisions would of necessity be reduction divisions and there would be no way in which the individuality of the chromosomes could be maintained from one generation to the next. I cannot, therefore, believe that the apparent pairing of the chromatin threads in the young spermatocytes of *Bufo* is of any great importance, although I do not question but that it may be a constant and important stage in the development of the germ-cells in other forms.

The condensation stage in the primary spermatocytes of *Bufo* is much more marked than in any of the other amphibians that have so far been investigated, and it is evidently a stage of relatively long duration, judging from the number of cases that are to be found in every section of the testis of adult toads killed during the summer months. The changes occurring in the chromatin during this time cannot, of course, be ascertained; but it is evident, from an examination of iron-haematoxylin preparations that have been considerably destained (Fig. 23), that the chromatin does not become a homogeneous mass. In light of the results obtained from the study of the maturation phenomena in the germ-cells of other forms in which there is no synizesis stage, or one much less marked than in *Bufo*, it would seem as if the final steps in the process of synapsis must take place at this time although I am very much inclined to believe that in *Bufo* this process may have had its beginning in the very young spermatocytes (Figs. 10, 11).

Whatever the changes taking place during synizesis, the chromatin emerges from this stage in the form of a continuous, homogeneous spireme. Although I have examined hundreds of spermatocytes at the stages of Figs. 24-28, I have never found the slightest indication of a longitudinal splitting of the spireme at this time. Such a splitting is first evident at the stage of Figs. 31-33, and then only in cases in which ring-shaped chromosomes are being formed.

If the chromosomes conjugated side by side during synapsis so that the opening in the ring-shaped chromosomes shown in Figs. 32, 33, 36, and 38 represents the space between two univalent chromosomes, as Montgomery has maintained, then the first maturation mitosis, which undoubtedly divides the tetrads formed from the rings in the same way that it does the dumbbell-shaped chromosomes, does not separate univalent chromosomes, but cuts each univalent chromosome in half and is, consequently, a reduction division. On the same assumption, the second division would be in the plane of the union of the univalent chromosomes and would separate the two remaining parts of each chromosome, being also a reduction division. Thus if we consider that the rings in the spermatocytes of *Bufo* are formed of two chromosomes *A* and *B*, which have conjugated side by side in synapsis as shown in diagram I, the first maturation division through the line *X* would cut each chromosome into two parts: *A* would be divided transversely into *a'* and *a''*; while *B* would be divided into *b'* and *b''*. The second division through the line *Y* would separate *a'* from *b'* in the one cell and *a''* from *b''* in the other cell. Both of the divisions being reduction divisions, the in-

dividuality of the chromosomes would not be maintained any more than if a double longitudinal division occurred. Wilcox (59) is, I believe, the only investigator who maintains that both of the maturation divisions are transverse, and his results have not been confirmed by those of other workers on insect spermatogenesis.

If, as seems more probable, synapsis takes place in the spermatocytes of *Bufo* by an end to end union of the chromosomes, the maturation divisions are diagrammatically represented by Diagram II. The first maturation division, through the line *X*, separates the univalent chromosome *A* from the univalent chromosome *B*, and is thus a reduction division. The second division, through the line *Y*, separates *A* into two equal parts *aa*, and *B* into *bb* and is, consequently, an equation

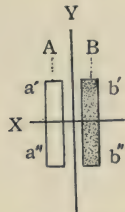


DIAGRAM 1.

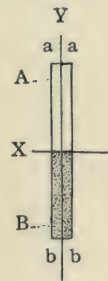


DIAGRAM 2.

division. It seems to me a matter of no great importance as to which mode of union takes place in synapsis provided that, in the subsequent mitoses, one division is reductional and the other longitudinal: the final result is the same in both cases. I agree fully with the conclusion reached by Grégoire (18), Farmer and Moore (12), and Montgomery (40), after an exhaustive résumé of the work done on the maturation phenomena in the germ-cells of animals and plants, that reduction occurs in the first maturation mitosis, the second division being longitudinal.

In none of the forms that have so far been studied is the evidence in favor of a reduction division in the maturation mitoses of the spermatocytes more convincing than in *Bufo*. The mode of formation of the dumbbell-shaped chromosomes in the early prophase of the first maturation mitosis and their later division across the middle of each dumbbell furnishes indisputable evidence that the first division is a reduction division in the Weismannian sense. That tetrads are normal structures in the spermatocytes of *Bufo* seems also indisputable. I have found tetrad groups in large numbers in the testes of adult toads killed at

various times during the summer months and also in the testes of young toads, so that in *Bufo* it is impossible that they can be "anomalies." The results of my work on *Bufo*, therefore, are in full accord with those obtained by vom Rath on *Rana* and *Salamandra* which have been ignored or severely criticised by other investigators of amphibian spermatogenesis.

The evidence brought forward by investigators who have studied the maturation phenomena in the eggs of amphibians has been overwhelmingly in favor of the occurrence of a double longitudinal division of the chromosomes previous to the extrusion of the polar bodies. Carnoy and Lebrun (9), and Lebrun (30, 31) have published a series of memoirs dealing with the formation of the polar bodies in the eggs of various amphibians, and they have emphatically denied the occurrence of a reduction division in the forms that they have studied. In my earlier study of the maturation of the egg of *Bufo lentiginosus* (King, 26) I was inclined to believe that both of the maturation divisions are longitudinal; but a later examination of a much more complete series of preparations of the first polar spindle (King (27)) led me to suggest the probability that the first maturation division is a reduction division. In light of the present study of the spermatogenesis of *Bufo*, and of investigations, not yet completed, on the ovogenesis of this amphibian, I am convinced that in the ovocytes as well as in the spermatocytes the first maturation division is a reduction division and that the second division only is longitudinal.

Although all investigators agree that the head of the mature spermatozoön is derived from the nucleus of the spermatid, there is no such unanimity of opinion regarding the origin of the other parts of the spermatozoön.

The middle-piece of the amphibian spermatozoön has been described as arising from the chromatin of the nucleus (Bühler, Flemming (16)) from the inner centrosome (Meves); from a part of the idiozome (McGregor) and from a nebenkörper (Hermann (20)). In the very young spermatids of *Bufo* the middle-piece anlage is frequently found some distance from the nucleus (Fig. 60), and there is therefore very little probability that it is a nuclear product. The appearance of the anlage of the middle-piece before the centrosome has divided precludes the possibility that it is derived from the inner centrosome. If, therefore, this structure is other than modified cytoplasm, it is very probably derived from the idiozome. As there is no structure in the spermatid at this time that at all resemble an idiozome, it must be that the entire

idiozome is transformed into the middle-piece and not merely a part of it as in *Amphiuma*. The results of my investigation of the origin of the middle-piece do not agree with those obtained by any other investigator of amphibian spermatogenesis, although they accord with Calkin's account of the origin of the middle-piece in *Lumbricus*.

As far as I am aware, Fick (13) is the only investigator who has found that the middle-piece of the mature spermatozoön of amphibians shows any great capacity for staining either with chromatin or with plasma stains. Fick states that the middle-piece of the spermatozoön of *Axolotl* stains intensely black after the use of iron-hämatoxylin, whereas in *Bufo* and in other amphibians it remains nearly colorless. This difference in staining reactions suggests that the inner centrosome in the *Axolotl* spermatozoön remains in the middle-piece and does not go into the posterior end of the head as it does in other forms.

The history of the centrosomes in the spermatid of *Bufo* is similar in many ways to that of the centrosomes in the spermatid of *Salamandra* and of *Amphiuma*, yet it differs in several important respects. In *Bufo*, as in the *Urodela* that have been most carefully studied, the axial filament of the tail grows out from one of the centrosomes while the other passes into the posterior end of the head. Whether this latter centrosome takes any part in the formation of the middle-piece, as Meves has stated for *Salamandra*, I have not been able to determine.

The history of the outer centrosome in the spermatids of *Bufo* is not easily traced as this body is small and loses its capacity for staining intensely at an early period. After the formation of the axial-filament, the outer centrosome wanders to the posterior end of the middle-piece, where presumably it remains. In the mature spermatozoön this centrosome probably serves as an "end-knob," as in the great majority of cases the connection between the two centrosomes, shown very clearly at the stages of Figs. 60-63, is broken before the spermatozoön becomes mature. I have never found the outer centrosome elongated as if it were in the process of division, neither have I found the ring-shaped structure around the axial-filament that is so conspicuous in the spermatids of *Salamandra* and of *Amphiuma*. The outer centrosome in *Bufo* behaves very much like the portion of the outer centrosome in the *Urodela* that remains at the posterior end of the middle-piece. This suggests the possibility that Hermann (21) is right in considering that the ring-shaped structure around the axial-filament in the spermatids of *Salamandra* is not derived from the centrosome. Herman states that the ring is formed from the mid-body of the last spermatocyte division;

but as Wilson (60) has pointed out, the figures given by McGregor and Meves show that the ring and the mid-body coexist in the young spermatid. Hermann's conclusion would seem, therefore, to be invalidated; but I can see no reason why the ring, if it is not derived from the centrosome, might not be formed from a part of the idiozome or, possibly, from a nebenkern.

There is as great a difference of opinion among investigators regarding the origin of the acrosome as there is concerning the formation of the middle-piece. Flemming (16) derives the apical portion of the spermatozoon of *Salamandra* from the achromatic substance of the nucleus; but this result is contradicted by the later researches of Meves (37) who finds, as does also McGregor, that this structure is formed from the idiozome. Broman (6) who has studied the formation of the spermatozoon in *Bufo igneus*, also derives the acrosome from the idiozome; but it is difficult to reconcile his description of the manner in which this formation takes place with that given by any other investigator of amphibian spermatogenesis. According to Broman, the idiozome becomes fixed at some point on the nuclear periphery and later the nucleus rotates in such a way that the idiozome is brought to the future anterior end of the spermatozoon. Whether the centrosomes, which are carried along with the idiozome, take any part in the formation of the acrosome is not stated.

Several investigators, among them Field (14), Niessing (42), and Platner (44), have maintained that the acrosome is formed from the spermatid centrosome; but such an origin of the acrosome has been denied by later workers on the same or on related forms, who have traced the centrosome into the middle-piece: Henking (19), Wilcox (59), and Paulmier (43), among others, have derived the acrosome in insects from a nebenkern; while Lenhossek (32) has described it in the rat as arising within the idiozome suddenly, as if it were a spontaneous thickening of the substance of the sphere from which it is later entirely separated, the sphere itself subsequently undergoing disintegration.

The young spermatids of *Bufo* contain in addition to the nucleus and the cytoplasm, a single centrosome surrounded by a clear vesicle, and a rounded, deeply staining body, the acroblast; there is no granular attraction-sphere in the cell, neither is there a nebenkern. As the acroblast is present in the cytoplasm of the primary spermatogonia and can readily be traced through all of the subsequent stages in the development of these cells, there is no possibility that it arises in the spermatid either from an idiozome, a mid-body, a centrosome, a nucleolus or a nebenkern.

Before the outer centrosome has reached the posterior end of the middle-piece (Fig. 63), the acroblast divides and one part migrates to the anterior end of the spermatid to form the acrosome. The fate of the acroblast that remains for a time in the posterior region of the spermatid I have not been able to ascertain. It is possible that this body has something to do with the formation of the marginal-filament or with the tail membrane. I cannot believe that it has persisted through all stages in the development of the spermatogonia unless it is to play some definite role in the formation of the spermatozoön.

An examination of the literature dealing with the spermatogenesis of amphibians shows that several investigators have found a homogeneous, rounded body in the cytoplasm that is similar in appearance to the acroblast in *Bufo*. Although Flemming and Meves makes no mention of such a body in the sperm-cells of *Salamandra*, Hermann states that in the spermatids of this amphibian "ein ovalärer Körper von dichter Consistenz findet sich; von einem hellen Hof umgeben." According to Hermann this body is formed in the spermatid from the remains of the achromatic spindle apparatus of the last spermatocyte division and soon disappears, being of secondary significance in the formation of the spermatozoön. Figures given by Kingsbury of the spermatogonia of *Desmognathus* show bodies in the cytoplasm resembling the acroblast, but no mention is made of these bodies in the text of the paper. The oval bodies found by Janssens in the spermatogonia of *Triton* are very like the acroblast in *Bufo*; but Janssens believes that these bodies are nucleoli extruded from the nucleus in order to nourish the cytoplasm. The behavior of the acroblast in *Bufo* would seem to invalidate the assumption that this body is an extruded nucleolus. As a rule, nucleoli that are ejected from the nucleus are dissolved at once or they lose their power of staining intensely and break up into granules which soon disappear. The acroblast, on the contrary, maintains its homogeneous appearance and stains very intensely during all stages of its development; it grows somewhat during the rest period of the cell, and then divides in the early prophase of mitosis, thus persisting as a definite structure in all generations of the sperm-cells.

In the cytoplasm of the spermatocytes and spermatids of various other groups of animals, small rounded bodies have been found that possibly are of the same character as the acroblast in *Bufo*. Since Benda (1) described the "chromatoid Nebenkörper" in the spermatocytes of mammals, a similar body has been found by other investigators of mammalian spermatogenesis. Lenhossek identifies the homogeneous body that is

present in the spermatocytes of the rat with the "chromatoid Nebenkörper" of Benda and he concludes, from its staining reactions, that it is an extruded nucleolus whose function may be to give up substances to the cytoplasm of the spermatid that are of use in the formation of the tail. Niessing also mentions the presence of a similar body in the spermatids of the guinea-pig, of the rat, and of the mouse. He traces this body back to the spermatocytes, although he fails to follow its history in the spermatids. Figures given by Niessing (Nos. 6, 21, 31, and 34) show that this body is in all respects like the acroblast in *Bufo*. His Fig. 34, showing a section of a young spermatid of a mouse, is especially interesting because in it this body is distinctly dumbbell shape, thus plainly indicating that it is in the process of division (compare with my Fig. 22, A). This stage given by Niessing is the one that I have been endeavoring in vain to find in the spermatids of *Bufo*; for although I have found numerous instances in which two acroblasts were lying close together, I have never succeeded in finding the actual division stage. It is a rather significant fact that Niessing is one of the few investigators who have stated that the acrosome is derived from a spermatid centrosome.

The acroblast in the young spermatids of *Bufo* might readily be mistaken for a large centrosome if one had not traced its development up to this stage. I am inclined to think, therefore, that the presence in the spermatids of other forms of a body similar in nature to the acroblast in *Bufo* may have caused many of the conflicting results that have been obtained by investigators regarding the origin of the acrosome. On this assumption it is probable that the "centrosome" described by Niessing, Field, Platner, and others as forming the acrosome, will be identified with the acroblast that migrates to the anterior end of the spermatid of *Bufo*; the real centrosome being found in the posterior end of the sperm-head or in the middle-piece. Platner's figures of the spermatids of a butterfly (Figs. 6-9) readily lend themselves to this interpretation, as his "centrosome" is much larger than that usually found in the spermatids of insects.

Besides the work of Platner, Henking's (19) figures of the spermatids of *Pyrrhocoris apterus* strongly suggest that a body like the acroblast may be present in the sperm-cells of some of the invertebrates as well as in those of the vertebrates. In addition to a nebenkern and a mitosome, both of which he derives from the remains of the spindle of the last spermatocyte division, Henking figures in the cytoplasm a small rounded, deeply staining body (Figs. 68, 69, 71, 72, 77-80). In the young spermatid this body seems to have no definite location, but in later

stages (Figs. 79, 80), it is found at the anterior end of the sperm-nucleus. In describing the origin of the acrosome, Henking states that the mitosome divides and that a portion of it becomes infected by an "un-zweifelhaft chromatisches Punktchen" so that the whole body stains like chromatin and then wanders to the anterior end of the spermatid to form the acrosome. Henking makes no mention in the text of his paper of the small bodies in the cytoplasm, and I am strongly inclined to think that his "infected mitosome" may prove to be an acroblast.

In this connection observations made by Foot and Strobell (17) are of interest. These investigators state that in the spermatozoön of *Allolobophora foetida* there are three centrosome-like structures, one at the base of the spine, one at the anterior, one at the posterior end of the middle-piece. It is perhaps possible that here, too, the centrosome-like body at the apex of the spermatozoön is derived from an acroblast.

In all of the cases in which a body similar to the acroblast in *Bufo* has been described or figured, this structure has the same characteristics: it is round or oval and somewhat larger than a centrosome; it always appears homogeneous and stains very intensely; it is usually surrounded by a clear area. It hardly seems as if bodies of unlike nature would have the same characteristics in the spermatids of such varied forms as the insects, the amphibians, and the mammals. Few of the investigators who have mentioned the presence of this body in the spermatids have traced it back to the spermatocytes; and none of them have followed its history in the spermatid. I feel confident that further research will show that in many, if not in all, of the cases mentioned above, the rounded, homogeneous body in the spermatids will be found to be of the same nature and significance as the acroblast in the spermatids of *Bufo*.

As the formation of the acroblast in the primary spermatogonia is as yet obscure, any conjecture I may make as to the origin of this body will be purely tentative. Judging from its staining reactions the acroblast is not extruded chromatin; and the behavior of this body lessens the probability that it is a nucleolus. I am strongly inclined to the opinion that the acroblast is a purely cytoplasmic product, formed possibly, from a condensation of a portion of the attraction-sphere at an early period in the history of the primary spermatogonia. If such proves to be the case, then in the spermatozoön of *Bufo* the acrosome has practically the same origin as the acrosome in the spermatozoön of *Salamandra*, *Amphiuma*, and *Bombinator*.

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## EXPLANATION OF FIGURES ON PLATES I-III.

All figures were drawn with the aid of a camera lucida under a Zeiss apoc. 1.5 mm., oc. 8. The following abbreviations are used in lettering the figures: *F. C.*, follicle cells; *A*, acroblast; *C*, centrosome; *Ac*, acrosome; *M. P.*, middle-piece.

## EXPLANATION OF PLATE I.

FIG. 1. Resting primary spermatogonium surrounded by follicle cells.

FIGS. 2-3. Early prophase of mitosis in a primary spermatogonium. All 24 chromosomes are shown.

FIG. 4. Equatorial section of the spindle in a primary spermatogonium during metakinesis.

FIG. 5. Longitudinal section of the spindle in a primary spermatogonium during metakinesis.

FIG. 6. A secondary spermatogonium during the resting stage.

FIG. 7. Early prophase of mitosis in a secondary spermatogonium.

FIG. 8. Longitudinal section of the spindle in a secondary spermatogonium during metakinesis.

FIG. 9. Anaphase of mitosis in a secondary spermatogonium.

FIG. 10. Young primary spermatocyte before the formation of the nuclear reticulum.

FIGS. 11-14. Growth stages of the primary spermatocytes.

FIG. 15. The primary spermatocyte at the end of the growth stage. The nucleus contains a spireme that appears to be continuous.

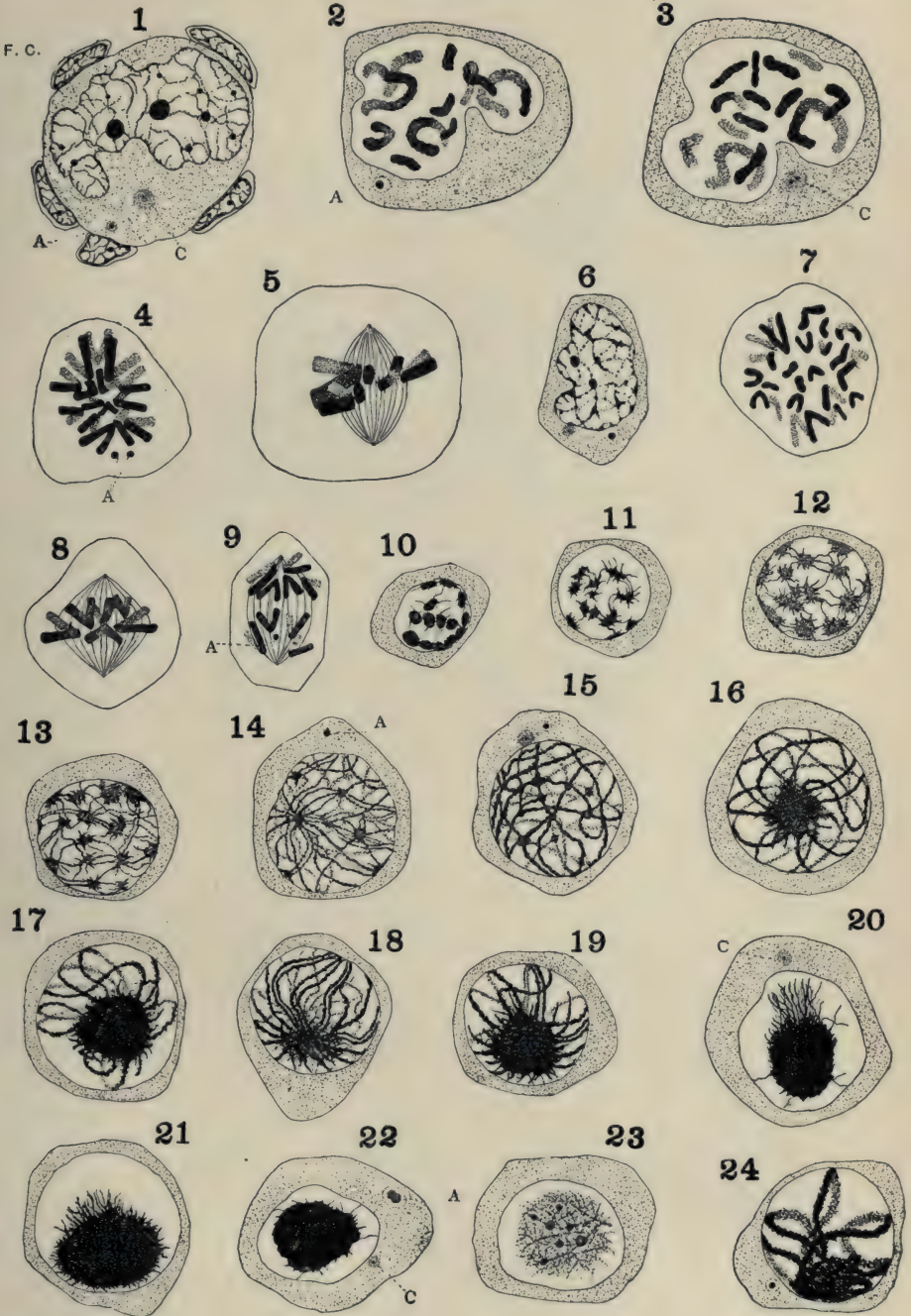
FIGS. 16-21. Stages showing the gradual condensation of the chromatin that precedes synizesis.

FIG. 22. Synizesis stage in the primary spermatocytes. The mass of chromatin is stained black with iron-hæmatoxylin.

FIG. 23. Same stage as the preceding, showing the appearance of the mass of chromatin when the greater part of the iron-hæmatoxylin has been extracted.

FIGS. 24-25. Post-synizesis stages in the primary spermatocytes. The chromatin is being evolved in the form of a continuous spireme.

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## EXPLANATION OF PLATE II.

FIGS. 24-25. Post-synizesis stages in the primary spermatocytes. The chromatin is being evolved in the form of a continuous spireme.

FIGS. 26-27. Continuous spireme found in the primary spermatocytes at the end of the synizesis stage.

FIG. 28. Beginning of the segmentation of the continuous spireme into the reduced number of chromosomes.

FIG. 29. A slightly later stage than that shown in Fig. 28. The spireme is being constricted into chromatin segments.

FIG. 30. The stage succeeding that shown in Fig. 29. The chromosomes are nearly separated and they are distinctly dumbbell shape.

FIGS. 31-33. Formation of ring-shaped chromosomes in the early prophase of mitosis.

FIG. 34. Primary spermatocyte in which the chromosomes are in the form of bead-like segments before they have separated. Such segments later form rings.

FIG. 35. Primary spermatocyte in which the spireme has segmented into rectangular shaped blocks.

FIG. 36. Formation of ring-shaped chromosomes from the spireme in the early prophase of mitosis.

FIG. 37. Primary spermatocyte in the early prophase of mitosis. The nucleus contains dumbbell-shaped chromosomes that are connected end to end by linin fibers.

FIG. 38. About the same stage as Fig. 37. The nucleus contains both dumbbell and ring-shaped chromosomes.

FIG. 39. Later prophase of mitosis. Chromosomes of different shapes are scattered throughout the nucleus.

FIG. 40. Ring-shaped chromosomes in the nucleus previous to the formation of the first maturation spindle.

FIGS. 41-42. Stages in the formation of the first maturation spindle.

FIG. 43. Equatorial plate of the first maturation spindle showing 12 chromosomes of different sizes.

FIGS. 44-45. Longitudinal sections of the first maturation spindle during metakinesis.

FIG. 46. Same stage as the preceding. The chromosomes have formed typical tetrad groups.

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## EXPLANATION OF PLATE III.

FIG. 47. Metakinesis of the first maturation division. Chromosomes of different types on the same spindle.

FIG. 48. Same stage as the preceding, showing the great difference in the size of the chromosomes.

FIG. 49. Early anaphase of the first maturation mitosis.

FIGS. 50-52. Anaphase of the first maturation mitosis.

FIG. 53. Massing of the chromosomes at the spindle poles in the late anaphase of the first maturation mitosis.

FIG. 54. Secondary spermatocyte. Division of the centrosome preparatory to the formation of the second maturation spindle.

FIG. 55. Metaphase of the second maturation mitosis, showing an amorphous mass of chromatin at the equator of the spindle.

FIG. 56. Equatorial plate of the second maturation spindle. Only nine of the twelve chromosomes are shown.

FIG. 57. Early anaphase of the second maturation mitosis.

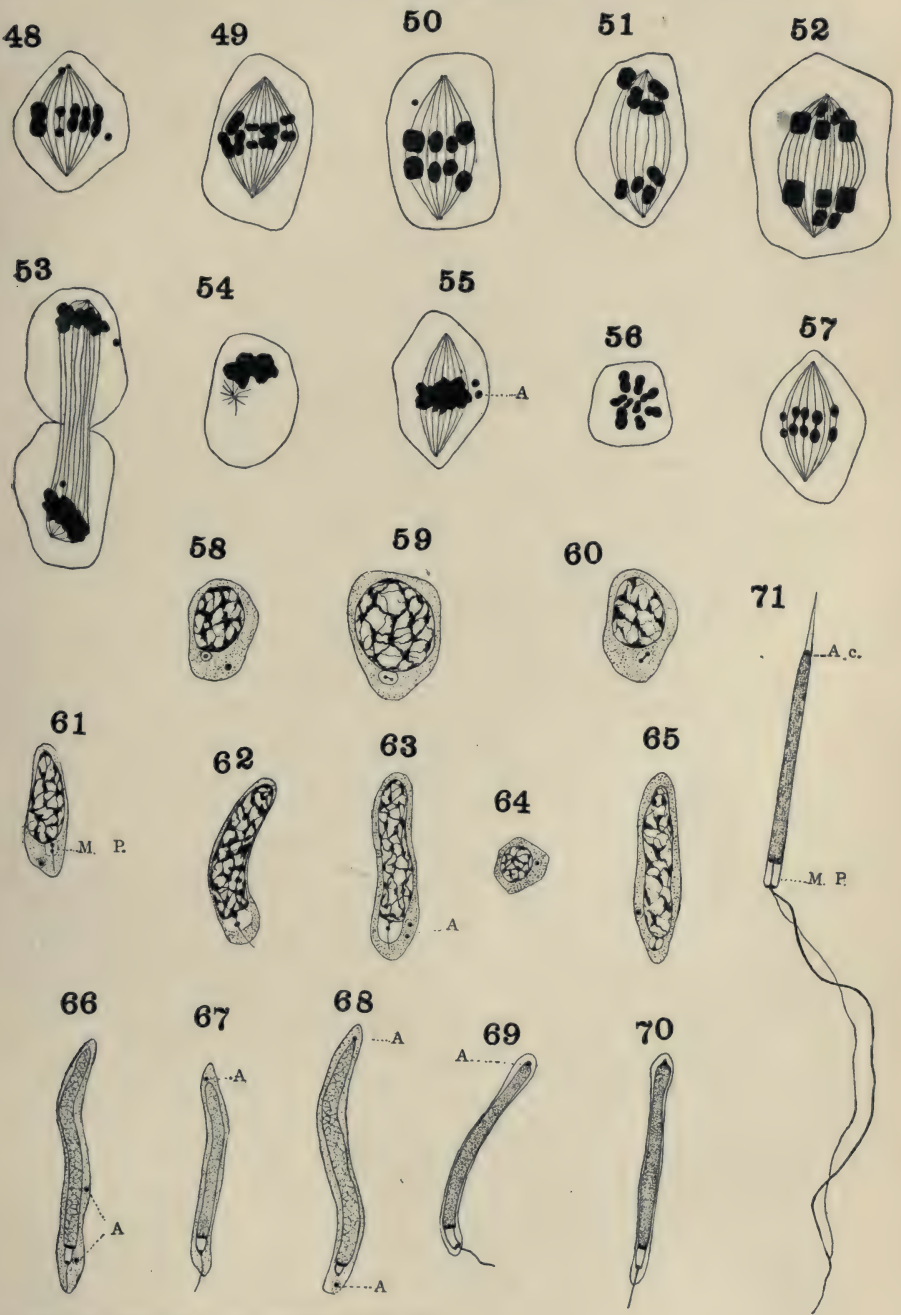
FIG. 58. Very young spermatid showing the single centrosome and the acroblast.

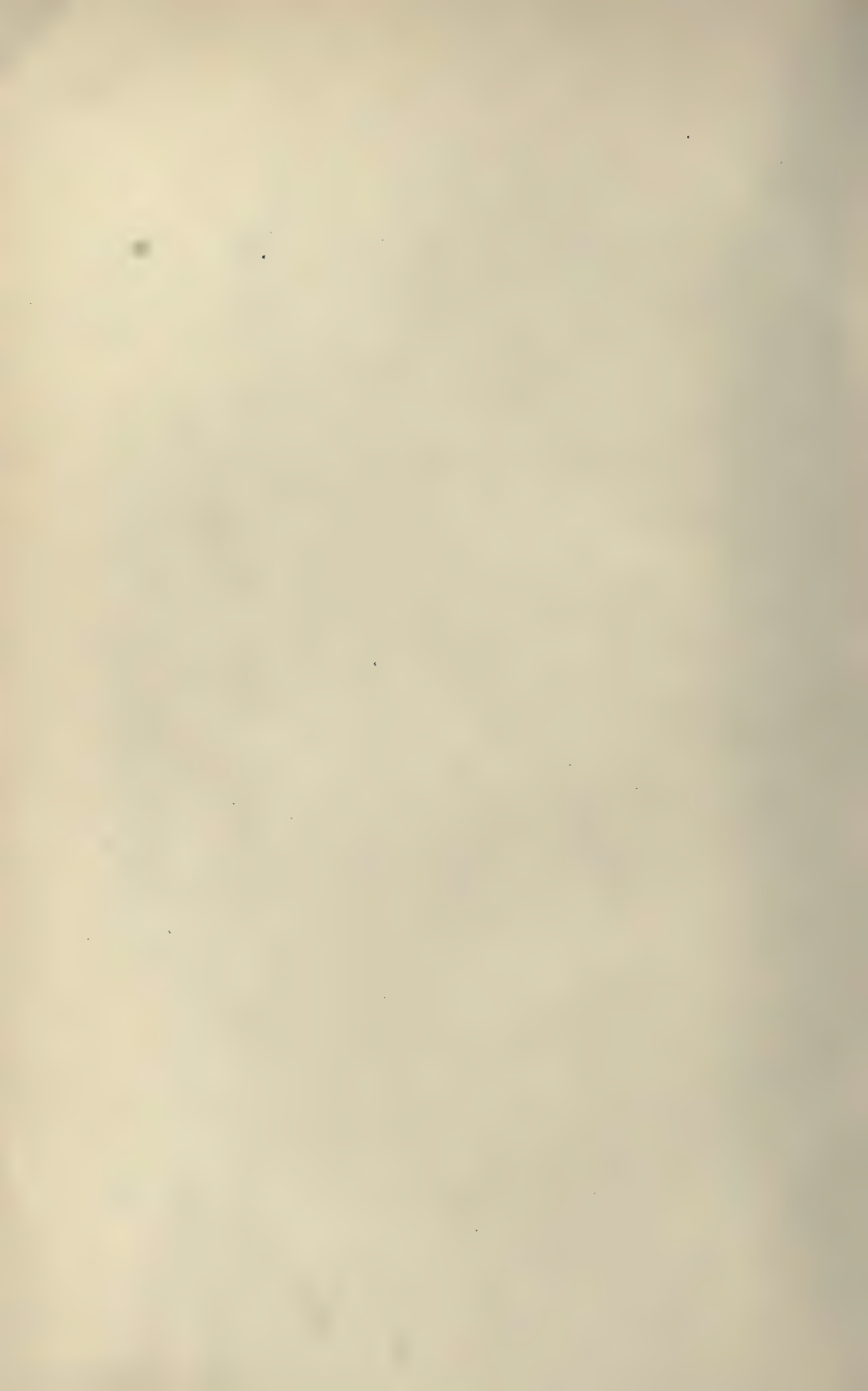
FIG. 59. Young spermatid. The centrosome has divided and the surrounding vesicle has come in contact with the nuclear membrane to form the middle-piece.

FIGS. 60-70. Stages in the development of the spermatid into the spermatozoön.

FIG. 71. Mature spermatozoön stained lightly with iron-hæmatoxylin.

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# THE INFLUENCE OF GRAFTING ON THE POLARITY OF TUBULARIA

BY

FLORENCE PEEBLES

WITH TWENTY-SIX FIGURES

The experiments made by Loeb, Driesch, Morgan, and others, have demonstrated that by closing the oral end of a piece of the stem of *Tubularia* the development of the aboral hydranth is hastened. The same result was obtained by Morgan ('03) when he bent long pieces in the middle, or ligatured them so that the *cœnosarc* of the two ends was completely separated. Morgan and Stevens ('04) have shown further, that the formation of a hydranth at the aboral end of a piece produces a change in that region, so that when this hydranth is removed, the piece is more likely than before to develop another aboral hydranth.

The object of the experiments described in this paper was primarily to determine what influence grafting exerts upon the polarity of *Tubularia mesembryanthemum*, but one experiment led to another until the investigations extended to a study of some of the factors of regulation.

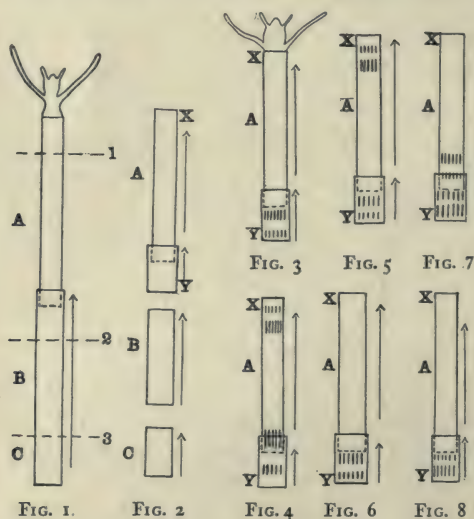
Last spring it was my privilege, through the generosity of the "Association for Maintaining the American Woman's Table at Naples," to spend two months at the Zoölogical Station, during which time I carried on the experiments described in the following pages. It gives me great pleasure to express here my gratitude to the Association, and also to Prof. Anton Dohrn, and the other members of the staff at Naples, for the courtesies extended to me during my stay.

In earlier experiments in grafting ('00 and '02) two components having the same diameter were selected, the two cut surfaces were applied, and held together until the *cœnosarc* united. This method proved so tedious that a new one was adopted. In

order to use this method one component must be slightly smaller than the other so that one end of the smaller one may be inserted in an end of the larger one. The two components were usually telescoped so that they lapped about one millimeter.

# I TWO LONG PIECES GRAFTED TOGETHER IN THE SAME DIRECTION

*Experiment 1.* The first series of experiments consisted in grafting together two pieces from the same region of two different individuals, so that the aboral end of one piece was inserted in the oral end of another piece from which the hydranth had just been



removed. Each piece measured about 3 cm. (Fig. 1), not including the hydranth of the distal piece which was not removed until the day after the graft was made. The first cut (1) removed the old hydranth and about 4 mm. of the stem from the distal component, the second cut (2) was made through the proximal component a short distance back of the line of union, and the last cut (3) removed the basal end from the proximal component. These three pieces (Figs. 1 and 2) I shall call respectively *A*, *B* and *C*. Their individual behavior after this second operation will first be considered, and then they will be compared in order to see the relative rate of development of the hydranths.

(*A*) The piece designated *A* (Fig. 2) consisted of the major part of the distal component, with a short distal piece of the proximal component grafted in the same direction, on its aboral end. If the long and short piece (Fig. 2, *A*) act as one, we should expect a new hydranth to form first at the oral end (Fig. 2, *X*) and later at the aboral end (Fig. 2, *Y*). If, however, the two components retain their individuality this result would not follow, for the oral end of the small component and the aboral end of the large component have had a start of twenty-four hours.

Forty-seven grafts were made, the results from these are given below in the table.

TABLE I A

Hydranths at <i>X</i> first, later at <i>Y</i> .....	18
Hydranths at <i>X</i> none, later at <i>Y</i> .....	14
Hydranths simultaneously at <i>X</i> and <i>Y</i> .....	6
Hydranths first at <i>Y</i> , later at <i>X</i> .....	6
Hydranths at <i>Y</i> , none at <i>X</i> .....	3
Total number of grafts.....	47

From this table it is evident that the oral end of the long piece is the region that produces the greatest number of hydranths, and that when they form at both *X* and *Y* (Fig. 2) they usually develop at the oral end of the long piece before they appear at the aboral end of the small piece (Fig. 3). About one-half of the hydranths forming at *Y* came entirely from the small piece, but in the reverse direction therefore they are aboral hydranths (Fig. 3). In the remaining one-half both components took part, the proximal row of tentacles appearing in the long piece, and the distal row in the short piece (Fig. 4). A large number of the grafts (almost one-third) developed neither hydranths nor stolons at *Y*. Six developed new hydranths simultaneously at *X* and *Y*. Of these, four of the aboral hydranths were composed partly of one, partly of the other component (Fig. 4), and two developed in the small piece only (Fig. 5). Twenty per cent of the grafts continued development at the line of union showing however the influence of the second operation, for the hydranths instead of taking their usual direction emerged from the cut end (Fig. 6). In those that

formed double hydranths, one at the aboral end of the long piece, and one on the short piece, the original direction of the small component was always maintained (Fig. 7). In one graft where the short piece developed a hydranth in the original direction (Fig. 8) it finally pushed the graft apart and emerged.

(B) Loeb ('04) has described a series of experiments on *Tubularia* in which he claims that the polarity was reversed. His experiment was as follows: A long piece cut from the stem was ligatured near the oral end. New hydranths developed at both ends. After the hydranths had emerged from the perisarc he cut a piece out of the region aboral to the ligature. Two days after the second operation ten pieces had formed aboral hydranths and only five had oral hydranths. From this result he concluded that the polarity was reversed. In other words the aboral end having formed a hydranth once, after the second operation, formed one again because the hydranth-forming material had been carried to that end.

In my experiment the piece *B* (Figs. 1 and 2) corresponds in position to the piece described by Loeb. Instead of waiting until a hydranth had formed at each end, the piece from which it was cut was united with another piece at its oral end and given a start of twenty-four hours at its aboral end. Some changes must have been going on at these two ends as some of the pieces did not behave like corresponding pieces cut immediately from stems that had not been grafted. The results are given in the following table:

TABLE 2 B

Oral hydranths not followed by aboral.....	23
Oral hydranths followed by aboral.....	15
Oral and aboral hydranths simultaneously.....	7
Lost.....	2
	—
Total number of pieces.....	47

The majority of pieces behaved like similar pieces in the control experiment, but in a few cases (seven out of forty-five) the development of the aboral hydranth was hastened. Not one piece developed a hydranth *first* on the aboral end.

(C) At the time of removal of the basal piece (Figs. 1 and 2) no sign of the tentacles' ridges was visible, and it was possible in only a few cases to see circulation at the aboral end. It does not follow however from this that there was no preparation for a hydranth. It was difficult to keep these short pieces oriented, therefore the results are meager. I have thrown out all those about which there was any doubt, so that the table gives a record of only twenty pieces.

TABLE 3 C

Hydranths at the aboral end first.....	8
Hydranths at the oral end first.....	6
Hydranths at the aboral and oral ends simultaneously.....	2
No hydranths.....	4
<hr/>	
Total number of pieces.....	20

On the eight pieces forming aboral hydranths first, the oral hydranths followed very quickly, a much shorter space of time intervening than between oral and aboral hydranths when the oral form first. These double ended pieces were kept until the hydranths dropped off, and a second set developed. In spite of the fact that those on the aboral end developed first, the second set appeared on the oral ends first, the aboral forming two days later. The pieces thus returned to their original polarity.

It is hardly possible to draw conclusions from such a small number of pieces, but the results are sufficient to show that the aboral hydranths after they are once developed do not exert enough influence over the region from which they come to establish permanently a marked polarity.

In order that we may compare one series as a whole, I have made the following table giving the relative time of formation of the hydranths on the pieces *A*, *B* and *C*. The region of the graft is indicated in *A* by the short horizontal line. The numbers placed beside the pieces show the order of emergence of the hydranths, the letter *S* stands for a stolon. Where no letter or number appear there was no regeneration. The arrows point toward the oral end of each piece.

TABLE 4

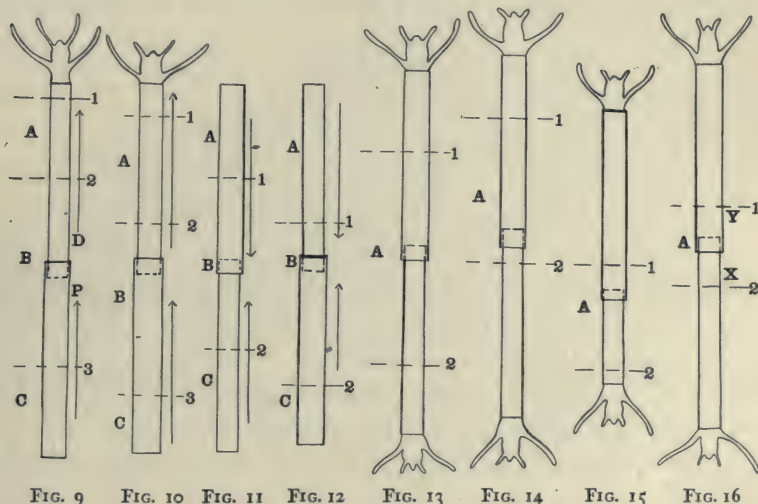
1	1	1	1	1	1	1	1	1	1	1	↑	A
						3	3				3	↑
2	2	2	1	2	2	2	2	1	2	2	↑	B
				3				3			3	↑
		2				2	2	2	2	2	↑	C
1	1			1	1	1	1				1	

This series of grafts shows the general result obtained from all. It will be noticed at once, that the oral hydranth on *A* forms sooner than that on *B* in nearly every case. The delay must be due to changes going on just in front of *B* at the line of union, for the pieces were originally from corresponding regions of the stems. The oral hydranth on *A* and the aboral on *C* appear at the same time, the oral hydranth on *B* and *C* at the same time, and the aboral hydranth on *A* and *B* at about the same time. The reason for this is obvious, for the aboral end of *C* had a start of twenty-four hours and the aboral surfaces of *A* and *B*, and the oral ends of *B* and *C* were exposed at the same time.

*Experiment 2.* A second series of experiments, somewhat similar to those just described, was made in order to find out (1) if the number of hydranths formed in the region of the graft would be increased if the two components were the same length, instead of one being much shorter than the other, and (2) to compare the rate of development on the oral and aboral ends of *A* and *C* (Fig. 9).

Two long pieces were united in the same direction, as described in the preceding experiment. After 24 hours the double piece was divided by three cuts (Fig. 9, 1, 2 and 3) but this time both the first and second cuts were made through the distal component, and the

third cut divided the proximal component in half. The piece *A*, between the first and second cuts, differed from *C* not only in position, but in time of exposure of the ends. Both ends of *A* were exposed at the same time, but the aboral end of *C* was exposed twenty-four hours before the oral end. In this experiment the graft is in the middle piece (Fig. 9, *B*).



Taking the piece *B* first as it is made of the two equal components grafted together in the same direction (Fig. 9) and comparing its later behavior with that of *A* in the first experiment, we find that the effect of the second operation is not so evident. Twenty-four hours after the piece had been separated from *A* and *C*, on 75 per cent of the pieces new hydranths were developing on the oral ends of the basal component, and on less than 50 per cent of the grafts oral hydranths were appearing on the distal components. The two components rarely acted as one piece, for one or more hydranths usually developed in the region of the graft. In the following table the results from twenty grafts are given. That part of the graft which came from the distal component is designated as *D*, that from the proximal component as *P* (Fig. 9).

If we compare Table 5 with Table 1, it will be seen at once that there is no marked difference in the number of hydranths formed

on each side of the line of union. A difference was observed in the number of aboral hydranths on the distal piece, although this is not shown in this table. Eight out of the twenty formed aboral hydranths in this experiment where the two components were of the same length, and in the first experiment where the proximal component was much shorter than the distal, only five out of twenty-seven, formed an aboral hydranth on the longer piece.

TABLE 5

Hydranths on the oral end of <i>D</i> .....	9
Hydranths on the oral end of <i>P</i> .....	11
Hydranths on aboral end of <i>D</i> .....	8
Hydranths on aboral end of <i>P</i> .....	4
<hr/>	
Total number of pieces.....	20

Before combining the results from the three pieces the behavior of pieces *A* and *C* (Fig. 9) will be considered very briefly. These two pieces were about the same length, but they were not taken from the same region of the original stems, and while the oral and aboral ends of *A* were exposed at the same time, those of *C* were not. The aboral end of *C* had a start of twenty-four hours. In Table 6 the results from twenty pieces are given.

TABLE 6

Oral hydranths.....	A 16	C 3
Aboral hydranths.....	A 2	C 15
Aboral stolons.....	A 1	C 0
No regeneration.....	A 1	C 1
		<hr/>
Total number of pieces.....		20

In order to compare the behavior of the three pieces Table 7 was made showing the results from twenty-one of the double components. In these grafts the second operation followed the first after a period of twenty-four hours.

*Experiment 3.* In a third series of experiments, instead of allowing the grafts to remain twenty-four hours before the second operation, the time was shortened, and after three to four hours, the pieces were isolated. The results are given in Table 8.

These results show a great difference in the region of the line of union. The number of hydranths formed there was greatly

TABLE 7

A

B

C

reduced, the double pieces resembling a single one in their behavior. Pieces of the same length as *A* and *C* were cut from corresponding regions of stems which had not been grafted. A com-

TABLE 8

Figure 1 consists of three schematic diagrams labeled A, B, and C, representing different types of 1D Ising models. Each diagram shows a vertical chain of spins (represented by numbers 1 or 2) and horizontal lines representing interactions between spins.

- (A) Nearest-neighbor interaction:** A vertical chain of spins. Horizontal lines connect adjacent spins, labeled with  $J$ . The spins are labeled 1 or 2.
- (B) Next-nearest-neighbor interaction:** A vertical chain of spins. Horizontal lines connect adjacent spins, labeled with  $J$ . Horizontal lines also connect spins that are two positions apart, labeled with  $J_2$ . The spins are labeled 1 or 2.
- (C) Potts model:** A vertical chain of spins. Horizontal lines connect adjacent spins, labeled with  $J$ . The spins are labeled 1, 2, or 3.

parison of their behavior with that of these pieces cut from the grafted stems shows a marked difference in the number of hydranths

developed. A much larger per cent of aboral hydranths formed on *A* in the control, and also more oral hydranths on *C* in the control. This seems to indicate that the process of hydranth formation at the grafted ends of these pieces affects their later behavior.

Table 7 and Table 8 show very definitely, that the results after the second operation are not modified by those following the first unless the period between the two operations is sufficiently long for them to get a start. In the first place (Table 7) the number of oral hydranths on the distal half of the original distal component, is much larger than that on the proximal half, while there are more aboral hydranths on the proximal half than there are on the distal half. This is not the case (Table 8) when the second operation follows the first after a very brief period. Secondly, the number of aboral hydranths on the proximal end of the original proximal component is much larger than the number on the distal half of the same component, and the number of oral hydranths on its distal half is greater than on the proximal half. This is not the case when the time between the first and second operations is reduced.

*Experiment 4.* In the fourth series of experiments the two components were grafted together in the way described above, but this time the level of the second cut was changed, making the piece *A* (Fig. 10) consist of the greater part of the distal component while *B* (Fig. 10) was made up of a short basal piece of the distal component grafted on the oral end of the proximal component. The third cut (Fig. 10, 3) was made nearer the aboral end of the proximal component thus making *C* shorter than in the preceding experiment. A series of sixteen grafts of this description are represented in Table 9.

If we compare the rate of development of hydranths on *A*, *B*, and *C* we find oral hydranths on *A* and *C* appearing at about the same time, also those on the oral and aboral ends of *B*. The per cent of hydranths on the oral and aboral ends of *A* and *C* was about the same as that in Experiments 1 and 3. The proportion of double hydranths at the region of the graft was larger, and also the number of aboral hydranths on the distal end of the proximal component. When this experiment is modified by decreasing the time

between the first and second operations (Table 10) no hydranths formed on the oral end of the proximal component in piece *B*, but a large number of the distal short pieces formed hydranths. A

TABLE 9

2 2 1 2 1 2 2 2 2 2 2 2 2 1 2 1 A

1 2 1 2 2 2 1 2 1 3 3 2 1 2 1 1 B

2 3 1 2 2 2 2 2 4 4 2 2 2 2 2 1 C

very small number of hydranths developed on the aboral ends of *A*, in this series not one. The oral hydranths on *A* and *C* appeared about the same time.

TABLE 10

## 2 TWO LONG PIECES GRAFTED TOGETHER BY THEIR ORAL ENDS

*Experiment 1.* Two pieces of stem, each measuring about 3 cm., were grafted together by their oral ends (Fig. 11). The grafts were left undisturbed for twenty-four hours, then the double piece was cut at two levels (Fig. 11, 1 and 2) so that each component was halved. The distal halves of each were united by their oral ends forming the piece *B* while the proximal halves of each (*A* and *C*) had their oral ends exposed by a fresh cut, their aboral ends having a start of twenty-four hours. New oral hydranths formed on *A* and *C* at practically the same time, the aboral usually preceding the oral by a few hours, or forming simultaneously with it. The piece *B* developed a very small proportion of hydranths at its free aboral ends, but in nearly every graft double heads formed, one on the oral end of each component; these emerged and finally pulled apart. These oral hydranths were much slower in developing than the oral hydranths on the free ends of *A* and *C*. When the second operation followed a few hours after the first, the percentage of hydranths formed at the line of union was greatly reduced. The free aboral ends rarely developed hydranths at the same time, one usually preceded the other by six or eight hours. The oral hydranths on *A* and *C* in this experiment formed before the aboral hydranths with very few exceptions.

*Experiment 2.* In a second series of experiments in which the two components were grafted together by their oral ends (Fig. 12), the level of the first cut was changed so that *A* consisted of the major part of one component, while in *B* instead of the components being equal in length, one was much longer than the other (Fig. 12, *A* and *B*). A period of eighteen to twenty-four hours elapsed between the first and second operations. Table 11 gives the results from eighteen grafts. These practically represent the entire series of experiments so that it is unnecessary to give other tables.

If we consider the rate of appearance of the hydranths we see that the percentage of aboral hydranths is very large, and that they appear before the oral hydranths of the same piece with few exceptions. The number of hydranths on the oral end of the

large component of the graft is greatly reduced, while a relatively large number form on the aboral ends of the smaller component. The pieces *A* and *C*, upon which the aboral hydranths appeared before the oral, were kept until the first hydranths were lost, and new ones developed. Without exception, the second set of oral hydranths appeared first, even in cases where the aboral hydranths had developed one to two days earlier than the oral ones. This shows without doubt that the polarity is not permanently reversed.

*Experiment 3.* In this experiment the second operation followed the first after six hours. The results show that the aboral ends had not had a sufficient start to produce hydranths before

TABLE II

The musical score is written on a five-line staff. The notation is a form of musical shorthand using numbers 1-5 and letters A, B, C. The staff has a central clef-like symbol. The notation is organized into three systems, each with a letter label (A, B, C) on the right. System A (top) has 15 measures. System B (middle) has 15 measures. System C (bottom) has 15 measures. The notation includes various rhythmic values indicated by the numbers and some accidentals (sharps and flats).

the oral ends. None of the grafts formed hydranths on their oral ends, instead, the majority developed a hydranth first at the aboral end of the short piece, and several hours later at the aboral end of the longer piece.

3 TWO LONG PIECES GRAFTED TOGETHER BY THEIR ABORAL  
ENDS

It has been demonstrated by many observers that a piece cut from the stem of *Tubularia*, if sufficiently long, shows marked polarity, i. e., a hydranth develops first on the oral end of the stem and then later on the aboral end. I have shown that when two





current has nothing to do with the order of appearance of the hydranths on the ends of the grafts. We must seek an explanation elsewhere. I believe that it requires a large amount of energy to construct a new hydranth. In order to produce sufficient energy certain metabolic processes are set up. These processes must begin as soon as the wound closes. If the condition of the stem is such that sufficient energy can be produced, hydranths are formed at once, if not, the development is delayed until there is enough energy. When two pieces are grafted together, some of this energy is expended in healing the wound, and uniting the pieces. If there is a large enough quantity left over, or already in the pieces, hydranths develop at once at the two oral ends, but if there is not a large enough amount present, one end is delayed until the hydranth has been completed. This hypothesis may serve to explain the hastening of the aboral hydranth after one has been formed or is about to form in that region. If the hydranth has formed there may be energy left over, if it is about to develop there is a large amount of energy present. Under normal conditions some stems contain more energy, or vitality. The preceding tables show that from the two original components a large number of hydranths may develop as many as eight, while from others only one or sometimes none, appear. The conditions of the experiment are apparently the same; the results can be explained in no other way than that one individual possesses more energy than another. I do not believe that there is any one material whose presence modifies the result, it is the state of all the materials at the time of the operation.

### 3 GRAFTING A SHORT DISTAL PIECE ON THE BASAL END OF THE SAME STEM

In an earlier paper ('00) I described a series of experiments in which a short distal piece of the stem was grafted in a reverse direction, on the proximal or aboral end of a long piece. The results which I obtained from a small number of experiments, seemed to indicate that the long piece influenced the rate of development of a new hydranth on the short piece, for the tentacle ridges on the short piece did not appear until after the hydranths had

emerged from the oral end of the longer piece, a region which was nearer the basal end than the short piece. The number of experiments was almost too small to warrant any definite conclusion. I have repeated this experiment a number of times and have finally come to the conclusion that the major component does not influence the minor one unless it shares in the formation of the hydranth which develops at that end.

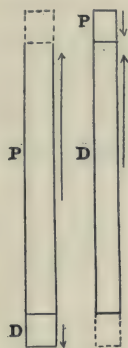


FIG 17 FIG. 18

*Experiment 1.* A short piece measuring about 1.5 mm. was cut from the distal end of a long (3 cm.) piece from a region near the hydranth. This small piece was then grafted on the aboral end of the long piece (Fig. 17) so that the oral end of each piece was exposed, the aboral ends united. Twenty-seven grafts are represented in Table 14. The dotted line shows the original position of the small piece.

TABLE 14

[illegible]

Eleven of the twenty-seven grafts formed a hydranth first at the oral end of the major component (*P*), and later at the oral end

of the minor component (*D*). Eleven developed oral hydranths on the long piece and nothing on the shorter one. Two developed hydranths on the short piece only, and two on the aboral end of the long piece only. In one case new hydranths appeared simultaneously on the two exposed ends. In all grafts where a long and a short piece are united, the formation of a new hydranth is always slower in the short piece. In six of the eleven grafts that developed the hydranth first at the oral end of the major component, the hydranth that formed later on the other end came partly from the long piece and partly from the short one, i. e., the distal tentacles developed in the minor component, and the proximal in the major component. In these the development was always slower.

#### 4 GRAFTING A SHORT BASAL PIECE ON THE DISTAL END OF THE SAME STEM

In order to test the influence of a long distal piece on a short basal one, a second series of experiments was made. This time the hydranth was cut from a piece of stem measuring 3 to 4 cm. From the basal end of this piece a short piece (2 to 3 mm.) was cut off, and grafted in the opposite direction, on the oral end of the same stem (Fig. 18). The results from these experiments were not surprising. Here no influence seemed to be exerted by the major component, as the following table shows.

TABLE 15

↓ 1	1	1	1	1	1	1	2	2	2	2	1	2	P
↑ 1	1	1			1	1							
													D
	1		2	1			1	1	1	1		1	

No very definite conclusions can be drawn from this table, or from any of the other series in this experiment. The major component apparently took no part in the formation of the hydranth in the smaller piece. From constant observation of the behavior of grafts composed of a short and a long piece, I am inclined to believe that the size of the short piece has more to do with the rate

of regeneration than contact with the major component. The only cases where it seems to me we are justified in looking for the influence of one upon the other is where the hydranth is developed partly in the long piece and partly in the short one. This did not take place in any of the experiments.

## 5 THE INFLUENCE OF THE CURRENTS ON REGENERATION AFTER GRAFTING

*Experiment 1. Two short pieces grafted in the same direction.* It was suggested to me by Professor Morgan, that the current in the two pieces of which a graft is composed, may have something to do with the order of regeneration in the outer ends of grafts. Soon after a piece is cut from the stem of *Tubularia*, the wound closes, and rapid circulation begins. The current is easily seen coursing up one side of the piece and down the other. When two pieces are united the currents do not always flow from one piece into the other. Instead, the current may be seen flowing up one piece and turning at the line of union as if stopped by a membrane, and continuing down the other side. Frequently, however, the current continues to flow up one side and on into the other piece. In Fig. 19 (*E* and *F*) these two cases are shown. In *E* the current is continuous; in *F* it is separate in the two pieces.

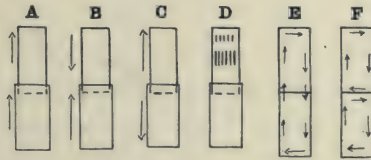


FIG. 19

Small pieces, measuring 1.5 to 2 mm. in length, were cut from different stems from a region at least 1 cm. back of the hydranth. One piece was inserted in the other so that the oral end of one overlapped the aboral end of the other (Fig. 19, *A*). At the end of twenty-four hours each graft was carefully examined under sufficient magnification to detect the direction of the currents. All of the grafts in which the circulation was continuous from one component to the other (Fig. 19, *E*) were put in lot *A*, while those in

which the circulation of one piece was distinctly separate from that of the other (Fig. 19, *F*) were isolated in lot *B*. Another lot (*C*) consisted of those in which the circulation was irregular, and the last (*D*) in which no circulation whatever could be detected. The rate of development of the hydranths is shown in Table 16.

TABLE 16

A			B			C				D		
↑	1	1	1	1	1	1	1	1	1	1	1	1
↑	2		2			2			2		2	

The behavior of the grafts is practically the same whether the circulation is continuous or not. In each case a hydranth developed first on one piece and then on the other or on one piece only.

*Experiment 2.* Two short pieces grafted together by their oral ends. Pieces of the same length as those in the preceding experiment were grafted together by their oral ends, so that the aboral ends were exposed. They were separated as before into lots *A*, *B*, *C* and *D*. The results from one series are shown in Table 17.

TABLE 17

A					B			C				D		
↓	1	1	1	1	1	1	1	1	1	1	1	1	1	1
↑	8			1	2	1	1	1	1	1	2	2		

Of these thirteen grafts four developed simultaneously on the aboral ends. Three formed one first on one end, then on the other, five formed one on one end and none on the other, and one developed a hydranth on one end and a stolon on the other. A control experiment was made in which single pieces, the same length as the entire graft, were cut. Out of eleven pieces, eight formed a hydranth on one end and nothing on the other; two developed a hydranth first on one end, and then on the other, and one developed a stolon on one end, and a hydranth on the other.

*Experiment 3.* Two short pieces grafted together by their aboral ends. These pieces were the same length, and taken from the same region of the stem, but were grafted by their aboral ends

(Fig. 19, *C*) so that the oral surfaces were exposed. A series of twenty-four grafts is represented in Table 18.

TABLE 18

A						B			C												D	
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	1	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2

Out of twenty-four grafts, twelve developed a hydranth first on one end, then on the other in spite of the fact that in some the current was continuous in the two pieces and not in others. Only one developed hydranths simultaneously on the free ends. Eleven formed a hydranth on one end only.

These experiments, as a whole, show that double pieces usually form one hydranth only (Fig. 19, *D*), or first one and then another later on the other end, regardless of the direction of the graft, or the flow of the currents.

## 6 THE EFFECT OF INTERRUPTING THE NORMAL PROCESS OF HYDRANTH FORMATION

Driesch ('97) first showed, in his researches on *Tubularia*, that when the formation of the hydranth is interrupted by separating the two tentacle ridges shortly after they appear, the method of completing the hydranth is not always the same. He has described four methods of regulation: (1) The "Regenerationsmodus" where the hydranth emerges with the original proximal tentacles, and later develops a new distal row; (2) the "Ersatzanlagemodus" where the coenosarc in front of the proximal row elongates and a new distal row appears before the hydranth emerges; (3) the "Auftheilungsmodus" where the proximal tentacles divide, forming the distal row from their distal ends; (4) the "Auflösungsmodus" where the proximal row disappears entirely and a new anlage forms. I repeated these experiments ('00) suggesting that the difference in the method of completion of the hydranth on the proximal piece was due to the degree of differentiation of the primordia. If the distal row of tentacles was removed soon after the red material had begun to collect in the two rows, the fourth method was invariably followed, i. e., the first proximal row dis-

appeared and the complete new anlage developed. If on the other hand the two rows were separated later after they were well defined, the first method of completion was followed. The proximal piece is, therefore, as Driesch has shown, capable of completing itself in a distal direction. The small piece (*A*) bearing the distal row of tentacles, is also able to complete itself distally, but as far as I am aware no one has found that such a piece is capable of forming new proximal tentacles, thus completing itself in a proximal direction.

It seemed to me worth while to repeat this experiment in order to find out at what time the distal piece (Fig 20, *A*) becomes so highly differentiated that it is no longer able to complete itself proximally, and also to observe the other methods described by Driesch, especially the "Auftheilungsmodus" which I had never seen, although I had repeated the experiment more than a hundred times.

In order to find out the exact time when the small piece (Fig. 20, *A*) becomes too highly differentiated to complete itself, it was necessary to remove the tip of the stem before the tentacle ridges were visible. Driesch tried this on forty-five pieces, allowing about twenty-four hours to intervene between the first and second operations. Out of these forty-five pieces, thirty-seven developed one row of tentacles, two formed a double row, and six a complete hydranth without a stalk. Five of the six pieces that formed a complete hydranth were "zu gross," therefore he considers that there were only three out of forty which developed more than the distal row. He concludes that there is therefore a definite time before the anlage appears, when the character of the further development of the smaller tip is determined. In order to determine the exact position of the tentacle ridges before they are visible on the outside, it was necessary to measure a number of anlagen, then to take the average length. Fifty pieces were cut from different stems, and left undisturbed until the anlage was visible on each. Measurements were then made, first from the tip of the stem to the base of the proximal tentacles (Fig. 21, *P*), and second from the tip of the stem to the base of the distal tentacles (Fig. 21, *D*) the average length of *P* was 1.7 mm. of *D* .6 mm.

*Experiment 1. Removal of the region of the distal tentacles before the ridges appear.* The hydranth and about 5 mm. of the stem below it were removed from twenty-seven pieces of stems whose length averaged 3.5 cm. These pieces were left undisturbed for eighteen hours. At the end of this time the circulation in the oral end was rapid but no ridges had been laid down. A piece .7 mm. long was then removed from the oral end of each piece (Fig. 22, *A*), thus separating the region in which the distal tentacles would appear later, (*A*) from the longer proximal piece (*B*) on which the proximal row would have developed. The pieces were isolated and their later behavior observed. The following table gives the results from twenty-five pieces, two of the twenty-seven having been lost.

## SERIES 1 A

Complete hydranth.....	5
Distal tentacles only.....	10
No regeneration.....	10
	—
Total number.....	25

By complete hydranth I mean distal and proximal tentacles, and reproductive organs. No stalk developed on these small pieces.

In the second series, twenty-two pieces were cut, the length of the distal piece (*A*) was increased from .7 mm. to 1 mm., thus including part of the region between the two rows of tentacles. The time between the first and second operations was the same as that in Series 1.

## SERIES 2 A

Complete hydranth.....	9
Distal tentacles.....	9
No regeneration.....	4
	—
Total number.....	22

Comparing Series 1 and 2, it will be seen that with an increase in the length of the distal piece (*A*) there is an increase in the proportion of complete hydranths.

In a third series *A* measured .8 mm. This time the tip was removed as soon as the piece was cut from the stem, so that no time elapsed between the first and second operations.

#### SERIES 3 A

Complete hydranth.....	0
Distal tentacles .....	7
Double row.....	1
No regeneration.....	4
	—
Total number.....	12

In this series one of the "incomplete" structures, i. e., a hydranth composed of two distal rows developed. This result is often obtained from short pieces. The piece *A* in this experiment was shorter than the short pieces from which Child ('07b) obtained a complete hydranth.

In a fourth series the anlage of the proximal row of tentacles was just visible as a faint red area. The length of the piece removed was .6 mm. The time between the operations was forty hours. The following table gives the results from thirty pieces.

#### SERIES 4 A

Complete hydranth.....	0
Distal tentacles.....	25
No regeneration.....	5
	—
Total number.....	30

Here no complete hydranths developed, while twenty-five formed the distal row which was probably already laid down when the two pieces were separated.

In a fifth series the piece *A* was removed immediately. This time the tip cut off exactly equaled the average area of the entire anlage (Fig. 23, *P*).

#### SERIES 5 A

Complete hydranth.....	12
Distal tentacles.....	0
No regeneration .....	3
	—
Total number.....	15

This table shows that the small piece if isolated before any of the processes preparatory to hydranth formation are begun, is not only capable of forming a complete hydranth, but in a large proportion of cases it does form the complete structure. The length of time between the operations and also the size of the tip removed are factors in determining the extent of later regeneration in the end of the piece.

The later behavior of the proximal piece (Fig. 20, *B*) is given in the following parallel series where the tip was removed before the ridges appeared.

SERIES 1 B

Old proximal row retained, new distal in front.....	10
New anlage.....	15
	—
Total number.....	25

It will be seen upon comparing this series with Series 1 A in each there were ten pieces that retained the original tentacles that had been laid down; these ten pieces were parts of the same piece before the second operation. The fifteen proximal pieces in Series 1 B which formed entirely new primordia, developed on their original tips five complete hydranths, while ten died without any further development.

SERIES 2 B

Old proximal row retained, new distal in front.....	9
New anlage.....	13
	—
Total number .....	22

Again it will be seen by comparing this series with Series 2 A that some of the proximal and distal pieces retained the original anlage which had been started before the second operation, the larger number, however, developed a new anlage. Series 3 and 5 B will not be given as the pieces developed new hydranths in the usual way.

In the fourth series, the second operation was postponed until after the proximal ridges were discernible, but the distal had not yet appeared.

## SERIES 4 B

Old proximal row retained, new distal in front.....	22
New anlage.....	8
Total number.....	30

The method of completion of the hydranth on the proximal piece (*B*) when the original row persisted, in Series 1 and 2 was that described by Driesch as the "Ersatzanlagemodus," a new distal row developed in front of the proximal row before the hydranth emerged. That in Series 4 was Driesch's "Regenerationsmodus" where the hydranth emerged first, and later a new row of distal tentacles developed. I agree with Child ('07c) that there is no essential difference in the two methods.

## CONCLUSIONS

These experiments prove that before the ridges have become visible, changes have taken place in the tip of the stem that render it when isolated incapable of producing a complete hydranth. These changes however do not take place until several hours after the wound has closed. If the tip is removed shortly after closure of the wound, even as long as twenty-four hours after the first operation, it is still possible for the isolated tip to form a complete hydranth. After the proximal tentacle anlage has appeared the distal piece when isolated, even if the tentacle ridges are not visible, does not form a complete hydranth. Instead the distal tentacles and mouth develop.

## 7 REVERSING THE DISTAL ROW OF TENTACLES

In earlier experiments ('00) I have shown that when the tip of the oral end of a long piece is cut off, reversed, and grafted back again at once, that the new hydranth develops in exactly the same region as it would have if the piece had not been removed. The distal row of tentacles appears in the small piece and the proximal row in the longer one. In the following experiment the tip was not removed until after the distal row of tentacles had appeared (Fig. 20). The piece *A* was then removed and grafted in

the reverse direction on the proximal piece (Fig. 24). This brought the distal tentacles into a position which was slightly different from their normal one. The space in front of them, i. e., between them and the end was greater than before. In a large number of experiments the pieces united, the tentacles completed themselves, and the normal hydranth emerged. In about 10 per cent of the grafts, a most interesting result was observed. The original distal tentacle ridges remained as distinct bands, while in front of them at the cut surface, a new row of distal tentacles and hypostome developed, the hydranth finally emerged with the stripes running from the base of the new distal tentacles to the proximal ones (Fig. 25). No reproductive organs formed on these pieces. The bands persisted until the hydranths dropped off. This serves to demonstrate that the small distal piece A when connected with the proximal piece is capable of developing new structures after the tentacles have been laid down. It also shows that the "red stuff" which is seen in the ridges, is not used again when a new row of tentacles is laid down. Stevens ('02) and Child ('07c) have observed in the proximal piece, after separation of the two ridges, that the "red stuff" which was originally in the proximal row forms a mass at the end of the stem, and when the hydranth is completed the mass is ejected.

#### 8 REMOVAL OF THE ENTIRE PRIMORDIUM

Driesch ('02) found when he removed the early anlage of the hydranth by a cut just below the proximal row, that in a large number of cases the ridges disappeared, and a new anlage developed, the latter being much reduced in length. Thus the length of the "reparation area" bore a definite relation to the length of the piece. Child ('07a) has also shown that there is a reduction in length of the primordia in short pieces, but the reduction in length is not proportional to the reduction in the length of the piece.

I have made a series of experiments in which long pieces were cut from the stems of different individuals, and after the tentacle anlage appeared, the distal end of the piece was removed by a cut below the proximal tentacles (Fig. 26, X). I hoped to find that at

a fixed distance below the primordium the stimulus of the cut would result in the formation of a short aboral hydranth while the oral ridges were fading out and the new short ones were appearing. The results were as follows: When the space below exactly equaled the length of the primordium (Fig. 26) the hydranth continued to develop without any sign of delay caused by the cut. No hydranth developed on the aboral end for this region became the stalk. When the space below the primordium was equal to one-half the length of the primordium the result was the same. The cut was then made close to the base of the proximal row of tentacles. In about one-third of the pieces the original anlage disappeared and a new one much shorter than the first appeared. No aboral hydranths formed on these pieces.

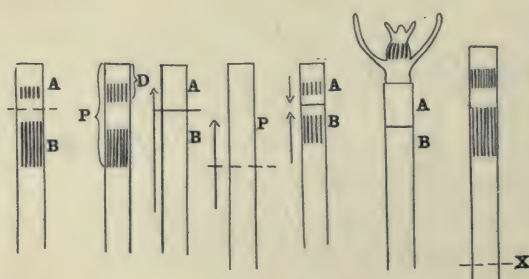


FIG. 20 FIG. 21 FIG. 22 FIG. 23 FIG. 24 FIG. 25 FIG. 26

## 9 THE INFLUENCE OF THE CONCENTRATION OF THE SEA-WATER

Loeb's earlier work ('92) on *Tubularia* brought to light the fact that the concentration of the sea-water is a definite factor in the regeneration of new hydranths. He found after testing various strengths, that sea-water diluted to 66 per cent was the optimum strength for growth. If the solution was weaker or stronger the growth was retarded. Snyder ('05) has also tested various strengths of sea-water, and has found that in *Tubularia crocea* when the sea-water was diluted not only was greater growth observed but a larger number of aboral hydranths developed.

My own experiments confirm those of Loeb and Snyder, but

some of the results which I obtained indicate that the great increase in size of the hydranths and the rapidity of their formation in dilute sea-water is due to something more than the difference in osmotic pressure.

The concentration of the sea-water in the Bay of Naples is estimated at 3.8 per cent. If this water is diluted to 2.5 per cent, growth is increased. Herbst ('04) found that artificial sea-water of the same strength as that of the Bay of Naples was more favorable for growth of sea-urchin larvæ than normal sea-water. I followed Herbst's formula and made a solution of artificial sea-water. I found that growth in this solution was more rapid and also that the hydranths formed in this solution were larger than usual, and lived longer. When the artificial solution was diluted with distilled water, the result was very different from that obtained from diluted water from the Bay. There was no increase in growth or in rate of regeneration. The results showed that the solution was not as favorable for growth as normal sea-water. I concluded from this that osmosis could not be the only factor in determining the increase in growth. It is not altogether improbable that organic substances in the Bay of Naples exert a retarding effect on growth. When these are excluded by preparing the pure artificial sea-water their retarding influence is abolished, and the growth which we consider so unusual is really no more than the normal rate under optimum conditions. This would explain why diluting artificial sea-water does not produce the same result as diluting that which comes directly from the Bay of Naples.

Child ('07c) has also made a study of the effect of diluting the sea-water. He concludes that the diluted medium increases the energy of the processes which involve hydranth formation. Since I made my experiments with dilute sea-water Child's work has been published. As my results are practically the same as his I shall omit a description of the experiments.

#### SUMMARY

1 When two individuals are grafted together changes at once take place in the region of the graft. These changes may not be

visible externally, but they exert some influence on the rest of the pieces, whether they result in the formation of new structures or not.

2 When the aboral end of a piece of the stem of *Tubularia* is stimulated through grafting to produce a hydranth before the oral end, the change in the polarity is not lasting for when a second set of hydranths develop, the piece assumes its original polarity.

3 When a short and a long piece are grafted together the influence of the longer piece is shown only when the new hydranth comes from the two pieces, a row of tentacles forming in each.

4 Short pieces grafted together in any direction usually develop a hydranth on one of the free ends only, or first on one end and later on the other. The result is the same whether the currents in the two pieces are continuous or not.

5 If the tip is removed from the end of a stem on which a new hydranth has just begun to develop, before the ridges are visible, the small piece is capable of forming a complete hydranth. If the tip is removed after the ridges are laid down, the piece develops one row of tentacles. The proximal piece completes itself distally by forming new tentacles on its tip, either before or after emerging from the perisarc. If the tip is removed before the primordia appear the proximal piece usually forms new primordia without delay.

6 If, after the appearance of the primordia, the piece in which the distal row of tentacles develops is reversed and grafted back on the proximal row, the hydranth completes itself in the normal manner. Sometimes the distal piece develops a new row of distal tentacles in front of the original red ridges which persist after the hydranth emerges.

7 When the entire primordium is removed by a cut just below the base of the proximal tentacles, the ridges frequently fade out and a new primordium develops which is much shorter than the original one. A cut 2 to 3 mm. below the primordium does not affect its later development. Such pieces do not form aboral hydranths.

8 Diluting normal sea-water in which pieces of *Tubularia* are placed, increases the rate of growth and the percentage of new

hydranths formed. Artificial sea-water has the same effect, but when the artificial sea-water is diluted these favorable results do not follow.

Bryn Mawr, Pa.

December 27, 1907

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# A STUDY OF THE GERM CELLS OF CERTAIN DIPTERA, WITH REFERENCE TO THE HETERO- CHROMOSOMES AND THE PHENOMENA OF SYNAPSIS

BY

N. M. STEVENS

WITH FOUR PLATES ✓

## INTRODUCTION

In connection with previous work on the spermatogenesis of the Coleoptera ('05, '06), the germ cells of the common fruit-fly, *Drosophila ampelophila*, were examined in the autumn of 1906. The difficulties encountered in handling this material led to the study of the spermatogenesis of several other flies. The results will be presented in accordance with the following scheme:

### Calyptrate Muscidæ.

#### Muscinaë.

- 1 *Musca domestica*.
- 2 *Calliphora vomitoria*.
- 3 *Lucilia cæsar*.

#### Sarcophaginaë.

- 4 *Sarcophaga sarraciniaë*.

#### Anthomyiinaë.

- 5 *Phorbia brassica*.

### Acalyptrate muscidæ.

- 6 *Scatophaga pallida*.
- 7 *Tetanocera sparsa*.
- 8 *Drosophila ampelophila*.

#### Syrphidæ.

- 9 *Eristalis tenax*.

## METHODS

With this material it was found that the best results could be obtained from fresh tissue mounted in Schneider's aceto-carmine.

The testes (or ovaries) of adult flies were dissected out in physiological salt solution and immediately transferred to a drop of aceto-carmin on a slide. The cover-glass was pressed down with a needle to break the capsule of the testis and spread the cells. All excess of stain was removed with filter paper, and after ten or fifteen minutes, the preparation was sealed with vaseline. Such preparations may be studied to the best advantage after twenty-four hours, as the chromatin gradually acquires a deeper tint. They remain in good condition for several days, but are, of course, not permanent. The method has several advantages besides that of enabling one to examine a large amount of material in a limited time. The aceto-carmin fixes and stains instantly without the shrinkage incident to the usual treatment with fixing fluids, alcohols, xylol and paraffin, necessary in order to obtain sections. Then, one is able to study the whole cell with all of the chromosomes present and uncut, which is an obvious advantage for work of this kind. The chromatin stains much more deeply than any other cell element, but the achromatic structures are not always well brought out, and they have been omitted from most of the figures, as this investigation is concerned primarily with the heterochromosomes and the method of synapsis. In favorable preparations of this kind, with good light, it is possible to get as accurate camera drawings as from sections stained with iron hæmatoxylin.

#### RESULTS OF INVESTIGATION

##### I *Musca domestica*

In many respects the spermatogenesis of this fly resembles that of *Tenebrio molitor* (Stevens '05), *Odontata dorsalis* (Stevens '06) and the other Coleoptera which have an unequal pair of heterochromosomes. There are, however, no synapsis, synize-sis or spireme stages in the spermatocytes, nor are tetrads ever formed.

In the prophase of spermatogonial mitoses one finds five pairs of long slender chromosomes, the members of each pair either lying parallel to each other or twisted together (Fig. 1). The members of the additional unequal pair are usually separate

(Fig. 1,  $h_1$  and  $h_2$ ). Apparently a side-to-side pairing or conjugation of homologous chromosomes, with the possible exception of the unequal pair, occurs preliminary to each spermatogonial mitosis. The twelve chromosomes separate, and each divides longitudinally in metakinesis. Whether they pair again in the telophase or not until the prophase of another cell-division is not evident.

The heterochromosomes remain condensed and are found side by side during the whole growth stage, while the other chromosomes pass into a more or less diffuse condition (Fig. 2). In the prophase of the first spermatocyte mitosis there are five thick V-shaped chromosomes and a pear-shaped mass of chromatin which in metakinesis proves to be the unequal pair of heterochromosomes (Fig. 3). The V-shaped chromosomes all divide longitudinally and the larger and smaller heterochromosomes separate as seen in Fig. 4. In the interval between the first and second divisions a nuclear membrane forms, but the chromosomes do not change greatly. Figs. 5 and 6 show the two kinds of daughter nuclei, one containing the larger, the other the smaller heterochromosome. In the second spermatocyte mitosis the V-shaped chromosomes again divide longitudinally and the heterochromosomes divide as shown in Figs. 7 and 8, so that in all stages they are clearly distinguishable from the ordinary chromosomes. The resulting spermatozoa fall into two equal classes, dimorphic as to the heterochromosomes, as in similar cases among the Hemiptera and Coleoptera. In most of the flies studied there was no difficulty in finding oögonia in which the number and relative size of the chromosomes could be determined. Only one such was found in *Musca*, that shown in Fig. 9. Here a part of the chromosomes are still paired; others have separated, but the members of each pair of ordinary chromosomes are not far apart; while the two equal heterochromosomes are on opposite sides of the group ( $h$ ). Here again we have what may be regarded as a partial synapsis of homologous chromosomes. The relation of the heterochromosomes in the two sexes is the same as in many of the Coleoptera (Stevens '05 and '06) and the Hemiptera heteroptera (Wilson '05 and '06), an unequal pair (large and small) in

the male and an equal pair of large heterochromosomes in the female. An egg which is fertilized by a spermatozoön containing the smaller heterochromosome produces a male, while one which unites with a spermatozoön containing the larger heterochromosome produces a female.

Although there is no distinct synapsis stage visible in the development of the spermatocytes of *Musca domestica*, the method of synapsis is without doubt indicated by the side-to-side pairing of chromosomes of equal length in the prophases of both spermatogonial and oögonial mitoses. The final synapsis is a closer union of the homologous chromosomes, and the first spermatocyte division separates the members of each pair instead of dividing each chromosome as in the spermatogonia.

## 2 *Calliphora vomitoria*

The chromosomes in this species are similar to those in *Musca domestica*. Both members of the unequal pair of heterochromosomes are smaller, as may be seen in a spermatogonial metaphase (Fig. 10). Pairing of homologous chromosomes is also evident here. In the growth stage (Fig. 11) the heterochromosomes are associated with a plasmosome as in many species of Coleoptera. Two views of the metaphase of the first spermatocyte mitosis are shown in Figs. 12 and 13, and an anaphase in Fig. 14. Two metaphases and an anaphase of the second division appear in Figs. 15, 16 and 17. The equal pair of heterochromosomes in the female is clearly shown in two oögonial metaphases (Figs. 18 and 19). In this case we have further evidence of the side-to-side pairing of homologous chromosomes in the spermatogonia and oögonia.

## 3 *Lucilia caesar*

Only a few specimens of this species were captured and the series of stages is incomplete. No spermatogonial or oögonial metaphases were found. In the growth stage a pair of *m*-chromosomes is present with an enormous heterochromosome bivalent

(Fig. 20). The metaphase of the first spermatocyte division is shown in Figs. 21 and 22, and prophases of the two kinds of second spermatocytes in Figs. 23 and 24. The spermatozoa would evidently be dimorphic as in the other species.

#### 4 *Sarcophaga sarracinia*

The three species of Diptera whose spermatogenesis has already been described belong to the sub-family Muscinae, while *Sarcophaga* is a member of the sub-family Sarcophaginae. The number of chromosomes in *Sarcophaga* is the same as in the other species, 12 somatic and 6 reduced, and the heterochromosomes closely resemble those in *Calliphora*. The spermatogonial plate (Fig. 25) shows the 12 chromosomes paired, but separated ready for metakinesis, and one chromosome shows the division line. In the growth stage (Fig. 26) the pair of heterochromosomes comes out clearly in the midst of diffuse and irregular masses of faintly stained chromatin. In these flies the ordinary chromosomes become much branched or diffusely granular in the growth stage but do not unite to form a spireme of even width as in so many forms. Whether or not they unite end-to-end at any stage before or after synapsis I cannot say. A prophase and an equatorial plate of the first spermatocyte mitosis may be seen in Figs. 27 and 28, and the metaphase and anaphase in Figs. 29, 30 and 31. The polar views of the metaphase of the second mitosis (Figs. 32 and 33) of course show dimorphism as to the heterochromosomes ( $h_1$ ,  $h_2$ ). Equal division of all of the chromosomes follows as in the three preceding species. Figs. 34 and 35 were drawn from adjacent oögonia in metaphase to show the close longitudinal pairing of the chromosomes and their later separation before metakinesis. The equal heterochromosomes are usually found together in the middle of the plate and each one is evidently equivalent in size to the larger heterochromosome of the spermatogonia and spermatocytes (Figs. 25 to 33). Fig. 36 is from an ovarian follicle cell. The four figures, 25, 34, 35 and 36 show the pairing of homologous chromosomes in spermatogonia, oögonia and somatic cells.

5 *Phorbia brassica*

Only one male of *Phorbia* was obtained and only four stages drawn; but these indicate precisely the same conditions as in the other species examined. *Phorbia* belongs to the sub-family Anthomyiinae. Fig. 37, a growth stage; 38, a prophase; 39, a metaphase; and 40, an anaphase, show clearly the presence of an unequal pair of heterochromosomes resembling those of *Musca domestica*.

6 *Scatophaga pallida*7 *Tetanocera sparsa*

The chromosomes of *Scatophaga* and *Tetanocera* resemble each other so closely in number, form and behavior that they will be considered together. Fig. 41 is a spermatogonial prophase of *Scatophaga*; and Figs. 42 and 43, spermatogonial prophase and metaphase of *Tetanocera*. All show equally paired ordinary or V-shaped chromosomes and unequally paired heterochromosomes. Figs. 44 and 45 are prophase and metaphase of the first spermatocyte of *Scatophaga*, Figs. 46 and 47 the corresponding stages for *Tetanocera*. In both species it will be seen that there is a close resemblance between the paired condition of the chromosomes in the prophases of a spermatogonial division and of a first spermatocyte mitosis. In general the chromosomes were larger in the spermatogonia (Figs. 41, 42, 43) than in the spermatocytes (Figs. 44, 45, 46, 47), but frequently prophases of spermatocyte mitoses could be certainly identified as such only by the metaphases in the same cyst and the growth stages in the neighboring cysts. The only actual observable difference between the synaptic condition in the spermatocytes and the spermatogonia is the behavior of the pairs in the following mitosis: in the spermatogonia the members of the pairs separate in metaphase (Fig. 43), and each divides in metakinesis; while in the spermatocytes the members of each pair remain closely associated in metaphase (Figs. 45 and 47) and separate in metakinesis (Fig. 48), but do not divide until the

second spermatocyte mitosis, though they frequently show the preparatory split in the anaphase (Fig. 49). We have here an unusually clear demonstration of the essential facts of synapsis and reduction, together with the rather unusual phenomenon of conjugation of homologous chromosomes in cells outside the sphere of maturation. Prophases of the second spermatocyte mitosis in *Scatophaga* appear in Figs. 50 and 51, and metaphases in *Tetanocera* in Figs. 52 and 53. An oögonial prophase and anaphase are given in Figs. 54 and 55, and a late prophase for *Tetanocera* in Fig. 56.

These two species as well as the one following belong to the Acalyptrate Muscidae.

### 8 *Drosophila ampelophila*

*Drosophila* has been placed at the end of the list of Muscidae because of the peculiarities which occur in the behavior of its chromosomes and the difficulties which have been encountered in their interpretation. While in *Sarcophaga* all the stages necessary for a description of the behavior of the heterochromosomes of both sexes were found in the course of a few hours' work on perhaps ten or twelve preparations, satisfactory results in the case of *Drosophila* have been obtained only after a prolonged study extending over more than a year and involving the dissection and examination of some two thousand individuals. Sectioning the material has never given satisfactory results. Hermann's platino-osmic solution and Worcester's formal-sublimate gave the best fixation, but the division stages are so scattering that permanent preparations, even if good fixation were secured, seemed less practical than the aceto-carmin method, which is much quicker and gives clearer pictures of the mitotic phenomena when they are present.

Spermatogonial mitoses are not abundant, and cells in which perfectly clear equatorial plates can be studied are exceedingly rare. The chromosomes in prophase are paired and twisted together in such a manner that it has been impossible to make an intelligible drawing of them in an early prophase. In Fig. 57, a

late prophase, two small spherical chromosomes and four larger elongated ones are distinctly paired while the members of the unequal pair ( $h_1$ ,  $h_2$ ) are separated. For a long time it was impossible to be sure that an unequal pair was present, as foreshortening in the case of one chromosome ( $h_2$ ) was possible, but recently a comparatively large number of good spermatogonial plates has been secured in which the inequality in length of one pair is clearly demonstrated. No case has been found in which the members of this pair appeared to be equal. Figs. 58, 59 and 60 show exceptionally clear cases, and Fig. 61 shows a peculiar folding of the chromosome  $h_1$ , whose significance may be apparent as we proceed to consider the unequal heterochromosome bivalent of the first spermatocyte.

In *Drosophila* the heterochromosomes cannot be demonstrated in the growth stages of the first spermatocyte. In some sections from Hermann material stained with thionin the plasmosome ( $p$ ) and some of the chromosomes appeared as in Fig. 62 in cysts adjacent to the spermatogonial cysts. In later growth stages nothing definite, except the immense plasmosome, can be made out in regard to the contents of the nucleus. The earliest prophase of division is the appearance of the chromatin massed together, usually on one side of the nucleus, while the plasmosome may be in the middle or on one side of the nucleus (Fig. 63). In aceto-carmin preparations the chromosomes first appear in early prophase, scattered through the nucleus, faintly stained and irregular in outline (Fig. 64). The plasmosome may be broken up at this time or it may appear intact in the spindle. Figs. 65 and 66 are later prophases in which the chromosomes are completely condensed. The unequal heterochromosomes are  $h_1$  and  $h_2$ . Fig. 67 shows the three equal bivalents, and the unequal heterochromosome pair in its simplest form, in the metaphase of the first spermatocyte mitosis. Fig. 68 shows slight modifications of this form from other cells of the same cyst. The most common form of this pair is seen in Figs. 69 and 70, where there are two equal V-shaped elements and a third portion ( $x$ ) which in many cases looks like a separate element, and for a time the group was thought to be trivalent; i. e., made up of two equal V-shaped

chromosomes and a smaller odd chromosome. This belief was strengthened by the appearance of many metaphases and anaphases (Figs. 70, 71, 72) where the third portion of the figure ( $x$ ) seemed to be on the point of separating from the V-shaped element next to it. This opinion was not confirmed however by the composition of the spermatogonial or oögonial equatorial plates, nor was it possible to demonstrate with certainty a separate element corresponding to  $x$  in the polar plates of the first spermatocyte mitosis or in the second spermatocyte. Fig. 72 is one of several cases where the portion  $x$  seemed to be separated from the two other elements of the group, but the separation must have been only apparent, for one much oftener finds an anaphase like Fig. 73 where the separation of the heterochromosome group into two unequal parts is certain ( $h_1, h_2$ ). Sometimes the anaphase is like Fig. 74, where more or less spherical masses replace the usual V's of the heterochromosome group. Often all of the chromosomes except the smallest pair show in the metaphase that they are elongated and V-shaped (Fig. 75), and in late anaphases (Fig. 76) the elements of the two largest bivalents are usually divided and the daughter chromosomes separated, often crossed. Both here and in the second spermatocytes it is often difficult or impossible to distinguish the heterochromosomes from the others. In the telophase the chromatin forms a dense mass which loses none of its staining quality and is soon resolved into the already divided chromosomes of the second spermatocytes (Figs. 77, 78, 79). A greater or less degree of elongation together with twisting and fore-shortening makes it impossible to measure or even estimate with any accuracy the relative length of the chromosomes, so as to distinguish the two classes of second spermatocytes as to size of heterochromosomes. Figs. 78 and 79 are two equatorial plates from the same cyst where the corresponding chromosomes are probably  $a-a$ ,  $b-b$ , and  $h_1-h_2$ . All of the chromosomes divide in this mitosis.

The oögonial metaphases are perfectly clear, and four equal pairs of chromosomes are always present (Figs. 80, 81, 82). In the metaphase they are usually grouped in pairs, and in the prophase they are closely approximated and twisted. In fact this

prereductional pairing of homologous chromosomes was first noticed in the oögonia and ovarian follicle cells of *Drosophila*. An attempt was made to ascertain whether such a pairing occurs in embryonic cells. Very little evidence was obtained. In the prophase of one mitosis paired chromosomes were found (Fig. 83). Fig. 84 is the equatorial plate of a segmentation stage. In both cases the pairs appeared to be equal.

### 9 *Eristalis tenax*

A considerable number of these flies were captured on some late blooming mustard plants in October. The material was in exceptionally favorable condition, and a complete series of drawings was obtained. The outer wall, or capsule, of the testis was thinner and more permeable to fixing fluids than in most of the other species studied and it was therefore possible to work with both sections and aceto-carmin preparations. This fly belongs to the family Syrphidæ, but the chromosomes in most respects resemble those of the Muscidæ. The heterochromosome bivalent is different in form from that of any of the Muscidæ described above; it however consists of a larger and a smaller component united in a somewhat different way from the corresponding elements in *Drosophila*.

Among the spermatocytes, several follicle cells were found in mitosis; the chromosomes of one such is shown in Fig. 85. The spermatogonial chromosomes are paired in prophase but separate and form a flat plate in the metaphase as seen in Fig. 86, where the two heterochromosomes ( $h_1$ ,  $h_2$ ) are conspicuously unequal in size. In this form there is a distinct synizesis stage, as shown in Fig. 87, from a section of material fixed with Gilson's mercurio-nitric fluid and stained with thionin. The cysts in which this stage occurs border upon the spermatogonial region of the testis. The outline of the chromosomes is visible and in the next stage the chromosomes are distinctly bivalents. Later they become more diffuse, but do not appear to form an even spireme at any stage. Fig. 89 is a growth stage, showing the heterochromosome group ( $h$ ), a pair of  $m$ -chromosomes and the other chro-

mosomes in a loosely branched condition. Fig. 90 is an early prophase in which the heterochromosome pair is very compact and deeply stained, while the other chromosomes are granular and denser in some parts than in others. A later prophase (Fig. 91), from a section, shows the heterochromosome pair assuming the cross-shape which we find in the later metaphase. Fig. 92 is a polar view of the equatorial plate of the first spermatocyte; and Figs. 93 and 94, side views of the spindle to show the cross-shaped heterochromosome bivalent in two positions. Here the cross (Fig. 94), instead of having opposite arms equal, as in cross-shaped tetrads composed of equal elements, has one of the vertical arms longer. It is evident from Figs. 93 and 95 that the longer arm is the smaller heterochromosome, while the remainder of the cross is the larger member of the pair. The larger element is folded in the same manner as in *Drosophila* (Figs. 66 and 67) but the smaller element is attached by one end instead of by the middle as in *Drosophila*. The second spermatocyte mitosis proceeds as in the other forms and presents nothing of especial interest. Dimorphism of the spermatozoa is foreshadowed by the first spermatocyte anaphases (Figs. 96 and 97). In the female the clearest figures were obtained from ovarian follicle cells (Figs. 98 and 99). The pairs are equal and comparison with the spermatogonial chromosome group (Fig. 86) indicates that the equal heterochromosome pair is one of the two longest.

The general results for the nine species of flies are the same; i. e., an unequal pair of heterochromosomes in the male leading to dimorphism of the spermatozoa, and a corresponding equal pair in the female, each equivalent to the larger heterochromosome of the male; also a prereducational pairing of homologous chromosomes in the prophase of mitosis in spermatogonia, oögonia, and ovarian follicle cells.

#### DISCUSSION

##### *I Sex Determination*

So far as I know there is no published work on the heterochromosomes of the Diptera. The literature on the heterochromo-

somes in other orders of insects has recently been so fully discussed in a paper by A. M. Boring ('07) that it seems hardly necessary to go into the subject exhaustively here. The dimorphism of the spermatozoa resulting from the maturation of the male germ cells of the nine species of *Diptera* considered in this paper is of the same character as that described by the author for 36 species of *Coleoptera* (see note, p. 49, Stevens '06), and by Wilson ('05 and '06) for several species of *Hemiptera heteroptera*. The dimorphism is brought about by the presence in the spermatogonia and spermatocytes of an unequal pair of heterochromosomes, while in large numbers of other insects such dimorphism is due to the presence of an odd chromosome in the male germ cells. These flies have proved to be exceptionally favorable material for demonstrating the occurrence in the female germ cells and somatic cells of a pair of chromosomes, each equivalent to the larger heterochromosome of the male.

Here, as in similar cases previously described, it is perfectly clear that an egg fertilized by a spermatozoön containing the smaller heterochromosome produces a male, while one fertilized by a spermatozoön containing the larger heterochromosome develops into a female. The material does not, however, throw any further light on the question whether the dimorphic spermatozoa are themselves in some way instrumental in determining sex in these insects; or whether sex is a character borne by the heterochromosomes and segregated in the maturation of the germ cells of each sex. If the latter supposition is true, sex is probably determined by the dominant heterochromosome of the egg, and fertilization is selective as has been shown in previous papers (Wilson '05, '06; Stevens '06, p. 54; Nowlin '06; Boring '07).

The only hope of determining whether sex is a Mendelian character seems at present to lie in breeding experiments with forms that may be shown by cytological study to be favorable. It is probable that in some cases at least, other characters may be so correlated with sex that their behavior in heredity may throw light on the sex question.

As to the proportion of sexes in these flies, a few figures may be given for *Drosophila ampelophila*. In the autumn and winter

of 1906-07, *Drosophila* was bred in the laboratory on two kinds of food, grapes and bananas. As the flies were dissected for the cytological work, a record was kept of the numbers of each sex; 1551 were so recorded. Of these 759, or 48.94 per cent were males; 792, or 51.06 per cent females. The records of the grape-fed and the banana-fed flies were kept separately. The total number of grape-fed flies dissected between November 1 and March 19 was 787, 404 or 51.33 per cent being males, and 383 or 48.67 per cent females. The banana-fed flies between October 30 and December 3 numbered 764, 355 or 46.47 per cent males, and 409 or 53.53 per cent females. In the total number there were 2.02 per cent more females than males, in the grape-fed 2.66 per cent more males than females, and in the banana-fed 7.06 per cent more females than males. These differences are probably not significant, but if sex is a Mendelian character, the numbers for the two sexes should of course be equal unless food produces some discriminating effect on the development of either individuals or eggs of the different sexes. It has always been a noticeable fact that the banana-fed flies were larger and more robust than those fed on grapes; this however applies to both sexes. In mass cultures it is not possible to tell whether failure of many of one sex or the other to reach the adult stage in different cultures might account for the discrepancies in numbers observed with the two kinds of food.

Castle and his co-workers ('06 p. 772) found the sexes about equal in three families of the sixth inbred generation of a grape-fed series, and the remarks which follow the table indicate that they regard the normal proportion as near equality.

Monkhaus' results on sex in *Drosophila* seem not yet to be in print, except for a brief report in the Year Book of the Carnegie Institution.

An attempt was made to ascertain the normal proportion of the sexes for the adults of *Musca domestica*. When caught by hand 58.33 per cent were females, but when a wire trap baited with sugar or molasses was used, only 46.53 per cent were females. The results need no comment.

Cuénot states that the normal proportion of males to females

in *Lucilia cæsar*, *Calliphora vomitoria* and *Sarcophaga carnaria* is approximately equal, and his experiments show that neither amount nor kind of food given to the larvæ has any marked effect on the proportion of the sexes in the first or second generation, but here as elsewhere in such experiments the number of eggs that did not hatch is not noted, and this may be the critical point. It is evident that more experiments are needed in which the fate of all of the eggs of isolated pairs of flies is determined.

## 2 *Synopsis*

In the spermatogenesis of most insects synapsis involves an end-to-end union of homologous chromosomes, and tetrads of various forms are commonly found in the prophase of the first spermatocytes. In these flies no tetrads have been observed and as a rule nothing comparable to the synizesis, bouquet or spireme stages of other forms is apparent. In these respects the germ cells of the Diptera resemble the oögonia of *sagitta* (Stevens '03 and '05) and the male and female germ cells of the aphids (Stevens '05 and '06). In the oögonia of *Sagitta* the chromosomes pair side-to-side in an early stage, while in the spermatogonia of the aphids the pairing occurs as a prophase of the first spermatocyte mitosis. The indications are that in the flies the chromosomes are already paired side-to-side at the beginning of the growth stage (Figs. 87 and 88), but the pairs do not appear to unite end-to-end to form a spireme. In some cases the members of the pairs are perfectly fused in the prophase of the first spermatocyte (Figs. 3 and 27); in others the bivalents are clearly such in both prophase and metaphase (Figs. 44 to 46). The first spermatocyte division is without doubt reductional for both ordinary chromosomes and heterochromosomes.

Perhaps the most interesting point in the whole study is the pairing of the chromosomes in cells somewhat removed from the sphere of the reduction process. This was first noticed in the oögonia of *Drosophila*, and was also found to occur in the ovarian follicle cells, the spermatogonia and some embryonic cells. This is not an occasional phenomenon, but one which belongs to every

oögonial and spermatogonial mitosis. In many cases the pro-phases of spermatogonia and first spermatocytes resemble each other very closely, the members of each pair being twisted together in both. In the spermatocyte we get complete synapsis and reduction; in the spermatogonium only a foreshadowing of reduction, and abundant proof that synapsis is here a side-to-side pairing of homologous chromosomes, and the first spermatocyte division a separation of univalent chromosomes, and not a longitudinal or quantitative division of two chromosomes united end-to-end. The relation of the heterochromosomes to each other in synapsis varies greatly with differences in form and size.

One is tempted to suggest that if homologous maternal and paternal chromosomes in the same cell ever exert any influence on each other, such that it is manifest in the heredity of the offspring, there is more opportunity for such influence in these flies than in cases where pairing of homologous chromosomes occurs but once in a generation. Possibly experiments in cross-breeding of flies may bring out some interesting facts in heredity.

NOTE. A preliminary statement in regard to the chromosomes of *Drosophila* was made at the International Congress of Zoölogists in Boston, August 21, 1907. The question as to whether an odd chromosome or an unequal pair of heterochromosomes was present in the male cells was then unsettled.

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### DESCRIPTION OF PLATES

With the exception of Figs. 59, 65, 73 and 82 for which a Zeiss 1.5 mm. obj. was used, the figures were all drawn with a Zeiss 2 mm. oil immersion obj. and a Zeiss compensating oc. 12. The magnification was doubled with a drawing camera, and the figures were then reduced one-half, giving a magnification of 1500 diameters.

#### *Lettering on plates*

$h$  = a heterochromosome or a pair of heterochromosomes.

$h_1$  = the larger heterochromosome.

$h_2$  = the smaller heterochromosome.

$m$  = an  $m$ -chromosome (Wilson).

$p$  = plasmosome.

$x$  = middle part of  $h_1$  in *Drosophila*.

## PLATE I

### *Musca domestica*

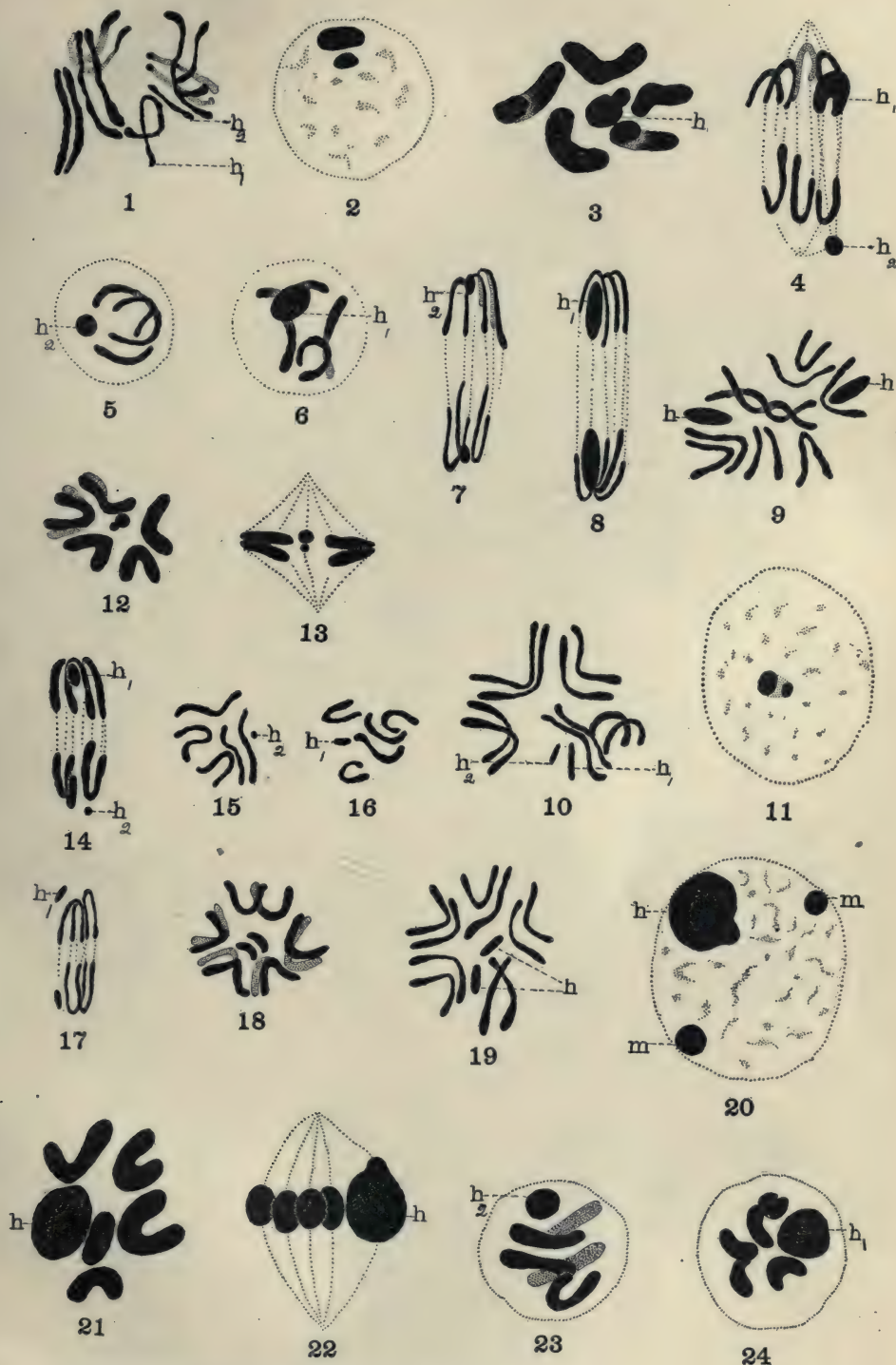
- Fig. 1 Spermatogonium, prophase, five equal pairs and one unequal pair of chromosomes.
- Fig. 2 First spermatocyte, growth stage.
- Fig. 3 First spermatocyte, prophase.
- Fig. 4 First spermatocyte, anaphase.
- Figs. 5 and 6 Second spermatocytes, prophase.
- Figs. 7 and 8 Second spermatocytes, anaphase.
- Fig. 9 Oögonium, metaphase.

### *Calliphora vomitoria*

- Fig. 10 Spermatogonium, metaphase.
- Fig. 11 First spermatocyte, growth stage.
- Figs. 12 and 13 First spermatocyte, metaphase.
- Fig. 14 First spermatocyte, anaphase.
- Figs. 15 and 16 Second spermatocyte, metaphase.
- Fig. 17 Second spermatocyte, anaphase.
- Figs. 18 and 19 Oögonia, metaphase.

### *Lucilia cæsar*

- Fig. 20 First spermatocyte, growth stage.
- Figs. 21 and 22 First spermatocyte, metaphase.
- Figs. 23 and 24 Second spermatocyte, prophase.



## PLATE II

### *Sarcophaga sarracina*

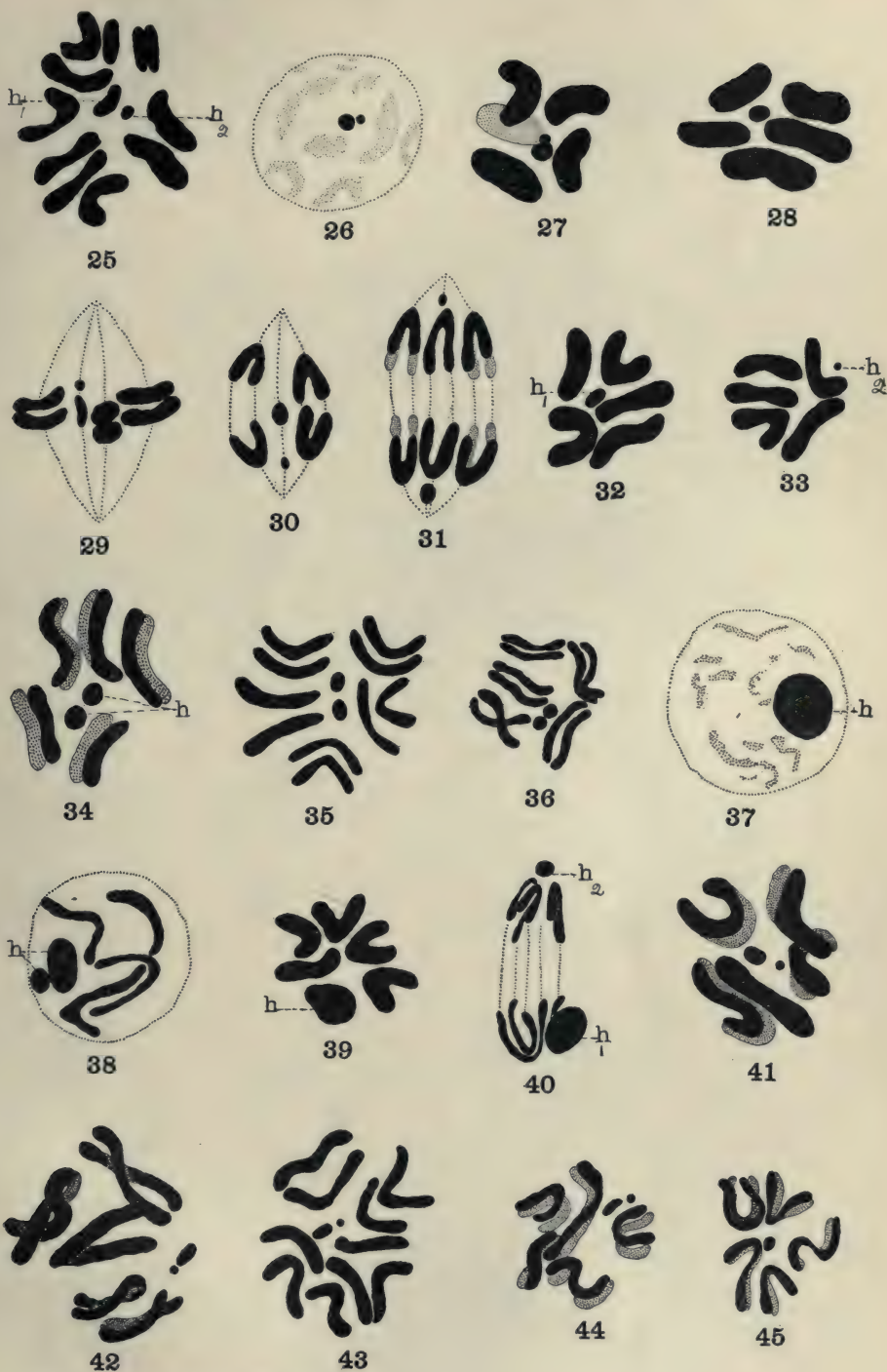
- Fig. 25 Spermatogonium, metaphase.
- Fig. 26 First spermatocyte, growth stage.
- Fig. 27 First spermatocyte, prophase.
- Fig. 28 First spermatocyte metaphase.
- Fig. 29 First spermatocyte, metakinesis.
- Figs. 30 and 31 First spermatocyte, anaphase.
- Figs. 32 and 33 Second spermatocyte, metaphase.
- Figs. 34 and 35 Oögonia, metaphase.
- Fig. 36 Ovarian follicle cell, metaphase.

### *Phorbia brassica*

- Fig. 37 First spermatocyte, growth stage.
- Fig. 38 First spermatocyte, prophase.
- Fig. 39 First spermatocyte, metaphase.
- Fig. 40 First spermatocyte, anaphase.

### *Scatophaga pallida* and *Tetanocera sparsa*

- Fig. 41 Scatophaga, spermatogonium, prophase.
- Fig. 42 Tetanocera, spermatogonium, prophase.
- Fig. 43 Tetanocera, spermatogonium, metaphase.
- Fig. 44 Scatophaga, first spermatocyte, prophase.
- Fig. 45 Scatophaga, first spermatocyte, metaphase.



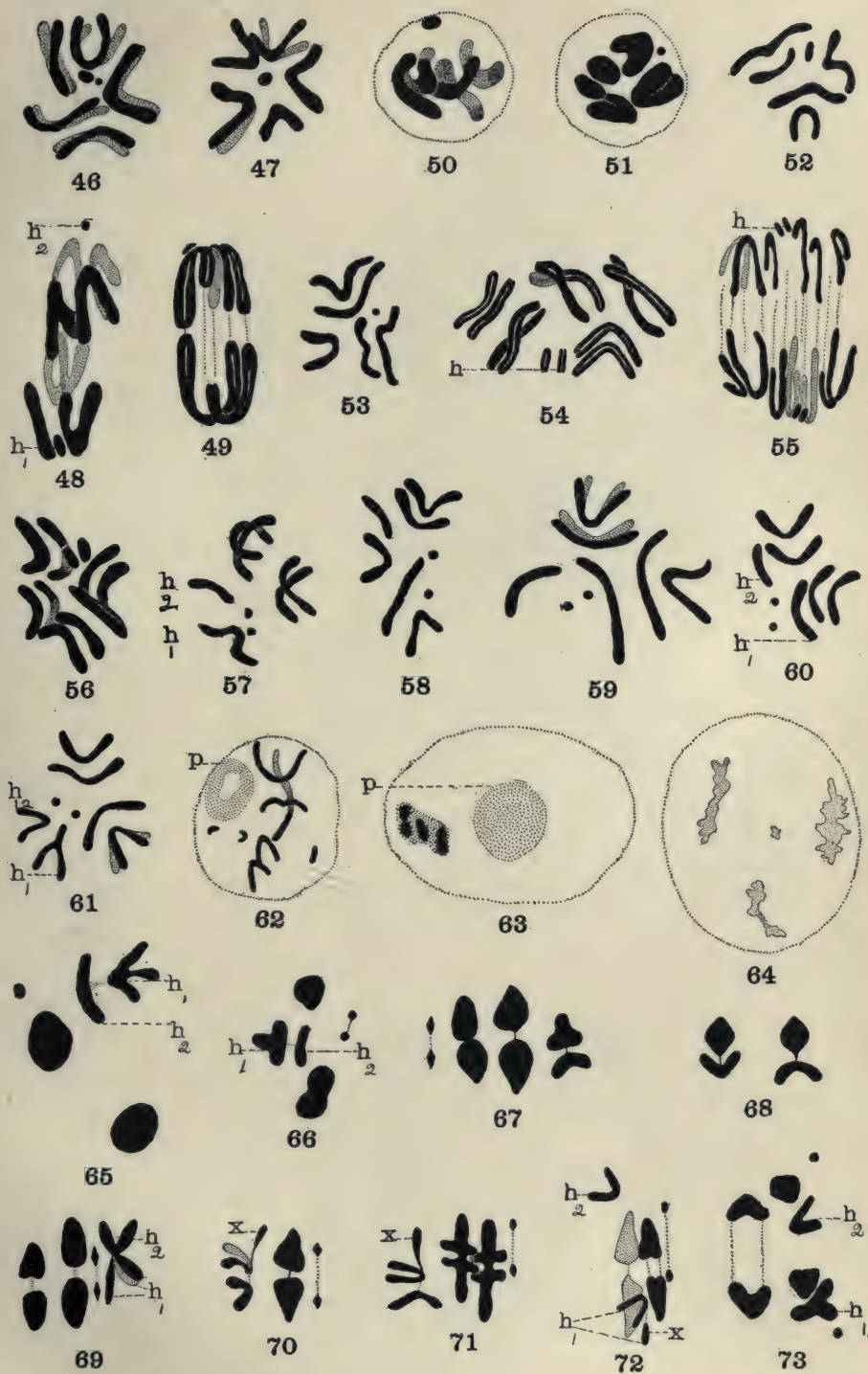
### PLATE III

#### *Scatophaga* and *Tetanocera* (continued)

- Fig. 46 *Tetanocera*, first spermatocyte, prophase.
- Fig. 47 *Tetanocera*, first spermatocyte, metaphase.
- Fig. 48 *Scatophaga*, first spermatocyte, anaphase.
- Fig. 49 *Scatophaga*, first spermatocyte, anaphase.
- Figs. 50 and 51 *Scatophaga*, second spermatocyte, prophase.
- Figs. 52 and 53 *Tetanocera*, second spermatocyte, metaphase.
- Fig. 54 *Scatophaga*, oögonium, prophase.
- Fig. 55 *Scatophaga*, oögonium, anaphase.
- Fig. 56 *Tetanocera*, oögonium, prophase.

#### *Drosophila ampelophila*

- Fig. 57 Spermatogonium, late prophase.
- Figs. 58-61 Spermatogonia, metaphase.
- Fig. 62 First spermatocyte, early growth stage.
- Fig. 63 First spermatocyte, very early prophase.
- Fig. 64 First spermatocyte, prophase.
- Figs. 65 and 66 First spermatocyte, late prophase.
- Fig. 67 First spermatocyte, metaphase.
- Fig. 68 Heterochromosome pairs.
- Figs. 69-71 First spermatocyte, metaphase.
- Figs. 72 and 73 First spermatocyte, anaphase.



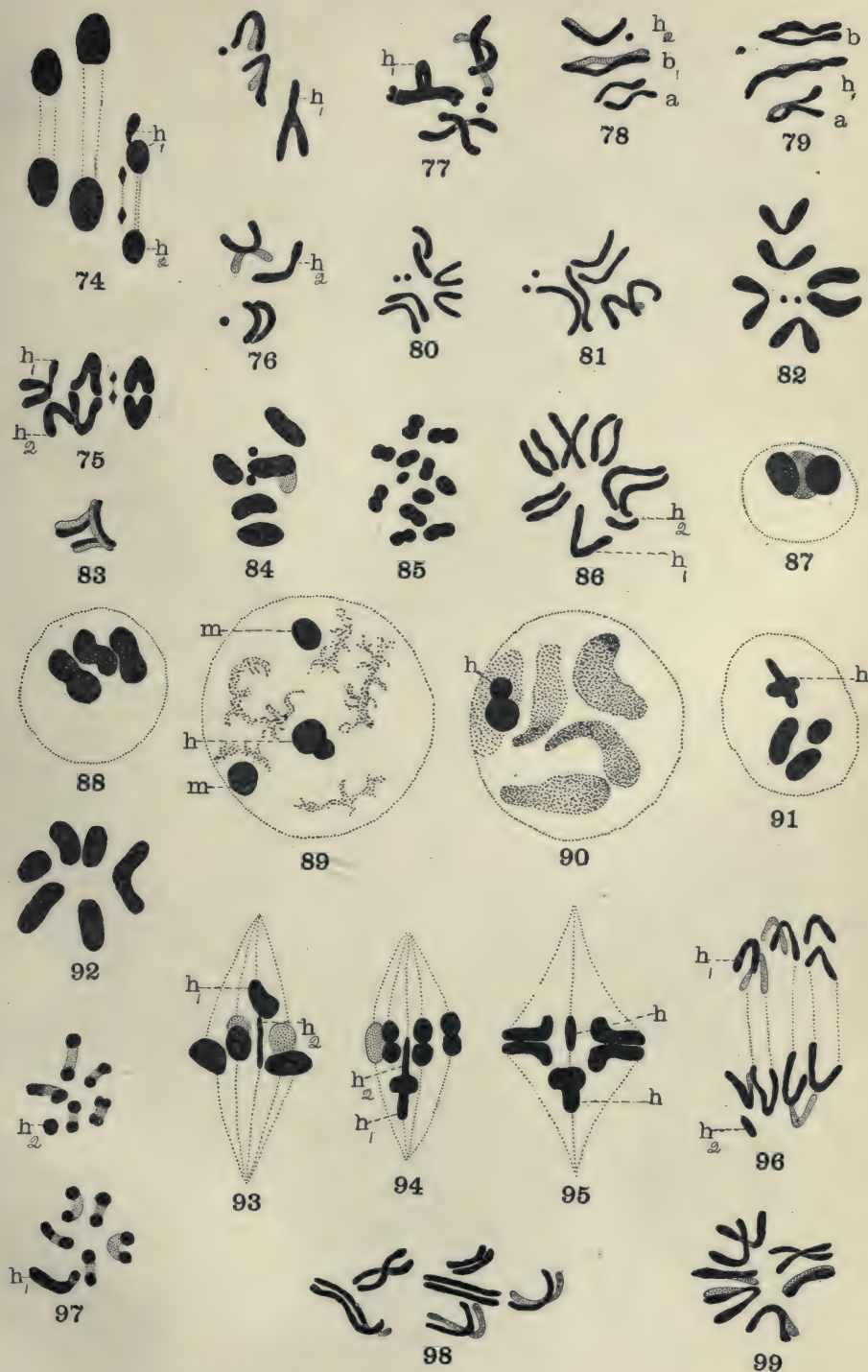
PALTE IV

*Drosophila* (continued)

- Fig. 74 First spermatocyte, anaphase.
- Fig. 75 First spermatocyte, metaphase.
- Fig. 76 First spermatocyte, anaphase.
- Figs. 77-79 Second spermatocyte, metaphase.
- Figs. 80-82 Oögonia, metaphase.
- Fig. 83 Chromosomes from embryonic cell.
- Fig. 84 Chromosomes from segmentation stage.

*Eristalis tenax*

- Fig. 85 Chromosomes of follicle cell of the testis.
- Fig. 86 Spermatogonium, metaphase.
- Fig. 87 First spermatocyte, synizesis stage.
- Fig. 88 First spermatocyte, growth stage immediately following synizesis stage.
- Fig. 89 First spermatocyte, later growth stage.
- Fig. 90 First spermatocyte, early prophase.
- Fig. 91 First spermatocyte, late prophase.
- Figs. 92-95 First spermatocyte, metaphase.
- Figs. 96 and 97 First spermatocyte, anaphase.
- Figs. 98 and 99 Chromosomes of ovarian follicle cells, prophase and metaphase





# THE CHROMOSOMES IN *DIABROTICA VITTATA*, *DIABROTICA SOROR* AND *DIABROTICA* 12-PUNCTATA

A CONTRIBUTION TO THE LITERATURE ON HETEROCHROMOSOMES AND SEX DETERMINATION

BY

N. M. STEVENS

WITH THREE PLATES

In Publication No. 36 of the Carnegie Institution of Washington, the spermatogenesis of a number of Coleoptera was described, and discussed with reference to the determination of sex. The study of the *Diabroticas* was begun at Cold Spring Harbor in the summer of 1906, and I wish to express my gratitude to Dr. C. B. Davenport for the privileges granted me both at the Carnegie Institution for Experimental Evolution and in the research laboratory of the Brooklyn Institute. I am also much indebted to Miss Isabel McCracken of Stanford University for material of *Diabrotica soror*, prepared with the greatest care and sent to me in December, 1906, and March, 1907.

The same methods were used as in previous work on the germ cells of the Coleoptera. The germ glands were fixed in Gilson's mercurio-nitric fluid, in Flemming's strong chromo-aceto-osmic solution, and in Hermann's platino-aceto-osmic fluid. Sections  $5\mu$  thick were stained with iron hæmatoxylin or with thionin. The aceto-carmin method was used for long series of *Diabrotica soror* and *Diabrotica* 12-punctata.

## *DIABROTICA VITTATA*

In the majority of the Coleoptera previously studied (85.7 per cent), an unequal pair of heterochromosomes was found. The *Diabroticas* have an odd or unpaired heterochromosome, resem-

bling in this respect the Lampyridæ and Elateridæ as well as many of the Orthoptera and Hemiptera.

In the spermatogonial equatorial plate of *Diabrotica vittata*, we find 21 chromosomes (Pl. I, Fig. 1) of various sizes and shapes. If  $x$  be considered the heterochromosome, the others can be mated, forming ten equal pairs. In sections stained with iron hæmatoxylin the division of the testis into several definite regions is very striking. The resting spermatogonia hold little of the stain, while the chromatin of the spermatocytes in synizesis and synapsis stages is very black, and again the spireme stage is pale. The synizesis stage here, as in several other Coleoptera (Stevens '06), appears to be a prolonged telophase of the last spermatogonial mitosis. Fig. 2 shows the appearance of the short, crowded chromatin loops in synizesis. Following this stage comes a period in which the chromosomes are uniting in synapsis, and one finds many nuclei similar to Fig. 3, some of the loops still short as in Fig. 2, others longer and showing a sharp angle or a knob at the point of union of two chromosomes. There is no such definite bouquet stage as in many forms, but one next finds a stage in which there are irregularly disposed loops with many free ends and some sharp angles like those in Fig. 3 (Fig. 4). In this stage the heterochromosome ( $x$ ) is for the first time evident, condensed against the nuclear membrane. This stage rapidly goes over into the spireme stage (Fig. 5), where all of the chromosomes except the heterochromosome ( $x$ ) seem to be united into a single spireme thread, and the points of union are no longer visible. The spireme is very pale and the heterochromosome therefore very conspicuous. There is nothing unusual in the prophases of the first division. The spireme segments and splits longitudinally, the daughter elements separate as in Fig. 6, then unite again and form rods, dumb-bells, V's and rings (Fig. 7). The chromosomes in the spindle (Fig. 8) are so attached to the spindle fibers that in meta-kinesis they separate into their univalent components, and go to the poles as short thick V's which mass together but soon separate for the second division without any definite rest stage. The unpaired heterochromosome ( $x$ ) is of course connected with only one pole of the spindle and does not divide in this division. Fig.

9 is the equatorial plate with the heterochromosome ( $x$ ) at a different level from the other chromosomes. Equatorial plates of the second division are shown in Figs. 10 and 11, the heterochromosome ( $x$ ) appearing in Fig. 10, and not in Fig. 11. All of the chromosomes divide in this division giving equal numbers of spermatids and spermatozoa containing ten and eleven chromosomes, respectively. The spermatids (Figs. 12 and 13) contain a chromatin nucleolus ( $n$ ), which is certainly not the heterochromosome, since it is found in all of the spermatids. As the head of the spermatozoön becomes more and more condensed, the nucleolus gradually decreases in size and finally disappears (Figs. 14 and 15). The ripe spermatozoön has a very long slender head (Fig. 16) which stains intensely black in contrast with the earlier gray stages (Figs. 14 and 15).

#### DIABROTICA SOROR AND DIABROTICA 12-PUNCTATA

*Diabrotica 12-punctata* of the eastern United States and *Diabrotica soror* of the Pacific coast states resemble each other so closely that one might easily be mistaken for the other. Both are greenish yellow or yellowish green with twelve black spots on the elytra. Kellogg describes *Diabrotica soror* as yellowish green and *Diabrotica 12-punctata* as greenish yellow. The color varies considerably with the age of the beetle. *Diabrotica 12-punctata* averages larger, shades more on the yellow, and the under side of the abdomen is green or yellow while in *Diabrotica soror* it is black. The color of the abdomen seems to be the one external character by which the two species can always be distinguished; for the size, ground color, and size and fusion of spots are extremely variable in both species.

A small amount of material of *Diabrotica 12-punctata* was collected at Bryn Mawr, Pa., in October, 1906. On examining the sections, it appeared either that the species was polymorphic as to its germ cells, or that there must be two or more sub-species or varieties, and possibly hybrids. It was too late to obtain more material of this kind, so, through the kindness of Miss McCracken, a supply of *Diabrotica soror* was secured for comparison with

the eastern species; and in the summer and autumn of 1907, 100 males of each species were studied by means of aceto-carmin preparations. The character of the chromosomes in the male germ-cells of the two species is precisely the same. About 50 per cent of the individuals examined have nine equal pairs of chromosomes and an unpaired heterochromosome, while the remaining 50 per cent have one, two, three or four additional small heterochromosomes.

#### DIABROTICA SOROR

##### *Type I*

The stages in the spermatogenesis of the first type are in most respects similar to those of *Diabrotica vittata*. The spermatogonial metaphase has nineteen chromosomes (Fig. 17), the unpaired chromosome ( $x$ ) being the largest. The synizesis and synapsis stages are similar to those of *Diabrotica vittata*, but less conspicuous in sections and the stages are less clear. The changes that occur between the telophase of the last spermatogonial mitosis and the pale spireme stage (Fig. 18) probably take place much more rapidly in this species. A polar view of the metaphase of the first spermatocyte division is shown in Fig. 19, a lateral view in Fig. 20, and a late anaphase in Fig. 21. The odd chromosome is usually found at or near one pole of the spindle in the metaphase (Fig. 20). The bivalents are similar to those of *Diabrotica vittata*, and the first division separates their univalent components. In preparations from Hermann material the chromosomes of the daughter plates (Figs. 22 and 23) often begin to show a vesicular condition and in telophase the heterochromosome ( $x$ ) forms a vesicle by itself, while the other nine chromosomes are blended together (Fig. 24). Fig. 25 is a later stage taken from a cyst in which some second spermatocyte spindles were present, while Fig. 24 was from a cyst containing a few first spermatocyte spindles. Half of the nuclei in these cysts of course contain no heterochromosome. The rest stage between the two divisions is more pronounced than in *Diabrotica vittata* where the chromosomes are simply massed together in telophase,

and separate for the second division without the formation of a nuclear membrane. The second spermatocyte equatorial plates are shown in Figs. 26 and 27, the heterochromosome ( $x$ ) appearing in Fig. 26. All of the chromosomes divide in this division, giving, as usual, two equal classes of dimorphic spermatozoa. The spermatids and spermatozoa are similar to those of *Diabrotica vittata*. The chromatin nucleolus is found in the earlier stages but is not visible in stages corresponding to Figs. 14 and 15, and the head of the mature spermatozoön is only about one-half as long.

### *Type IIa*

About two-thirds (33 out of 100 males collected at Mountain View, Cal.) of the individuals belonging to the second type have one additional small chromosome, making twenty in the spermatogonia (Pl. II, Fig. 28). The additional chromosome appears as a second heterochromosome in the growth stages (Fig. 29,  $s$ ). In the first spermatocyte spindle the larger heterochromosome ( $x$ ) is found, as usual, near one pole of the spindle, while the smaller one ( $s$ ) may be in the equatorial plate (Fig. 30) or on either side of it (Figs. 31, 32, 33), closely associated with  $x$  or as widely separated from it as possible (Figs. 33 and 31). Fig. 34 is a polar view with the two heterochromosomes near one pole of the spindle. The small chromosome may or may not divide in the first division. In some individuals it almost always (possibly always) divides as in Fig. 35 later than the other chromosomes. In other cases it may be found undivided between the daughter plates (Fig. 36), outside of one of them (Fig. 37), or it may be concealed in the general polar mass of chromatin. In the telophase and brief rest stage (Figs. 38 and 39) it is often quite distinct from the remainder of the chromatin. Whether it divides in this mitosis or goes undivided to one pole or the other seems to be a matter of chance, depending perhaps on the part of the spindle which it happens to enter in the prophase. It seems to be much less automatic in its behavior than the other chromosomes. This peculiarly erratic behavior of the small heterochromosome in the

first division gives, or may give, in the same individual six different kinds of second spermatocytes with reference to this chromosome ( $s$ ), while there are, as usual, two kinds with reference to the large heterochromosome ( $x$ ). If the small chromosome goes undivided to the same pole with the odd chromosome ( $x$ ) (Fig. 33), we have second spermatocytes containing nine and eleven chromosomes (Figs. 40 and 41); if it goes undivided to the other pole (Figs. 31 and 37), the resulting second spermatocytes each contain ten chromosomes, one showing the large the other the small heterochromosome (Figs. 42 and 43); while if it divides, the second spermatocytes contain ten and eleven chromosomes (Figs. 44 and 45). As might be expected one finds two conditions in the second spindle. Either a small daughter chromosome is found outside of the equatorial plate (Fig. 46), or the small chromosome which has not divided in the first division, divides in the second (Fig. 47). Both conditions may be found in the same cyst. It is, of course, in only a few favorable spindles that it is possible to see the small chromosome actually dividing, but the metaphases are readily separated into two classes, one where all of the chromosomes are in the equatorial plate (Fig. 48) and another in which one small chromosome, which from its form and size is evidently a daughter chromosome from the first division, appears outside of the plate and often quite near one pole (Fig. 46). It is therefore quite certain that the small heterochromosome divides in either the first or second division but not in both. Clear daughter plates of the second division have never been found.

The conditions described above lead to the production of two equal classes of spermatozoa with reference to the large heterochromosome ( $x$ ) and four classes, which may be quite unequal, with reference to the two heterochromosomes.

$$\text{Equal numbers} \left\{ \begin{array}{l} \text{I} \left\{ \begin{array}{l} 9 \\ 9 + s \end{array} \right\} \text{variable numbers.} \\ \text{II} \left\{ \begin{array}{l} 9 + x \\ 9 + x + s \end{array} \right\} \text{variable numbers.} \end{array} \right.$$

If  $s$  always went to the same pole with  $x$  in the first division the classes of spermatozoa would be as follows:

$$\text{Equal numbers } \begin{cases} \text{I} & 9 \\ \text{II} & 9 + x + s \end{cases}$$

If  $s$  always went to the opposite pole from  $x$ , we should get the following results:

$$\text{Equal numbers } \begin{cases} \text{I.} & 9 + s \\ \text{II.} & 9 + x \end{cases}$$

If  $s$  always divided in the first spermatocyte division, there would be four equal classes of spermatozoa:

$$\text{Equal numbers } \begin{cases} \text{I } \left\{ \begin{array}{l} 9 \\ 9 + s \end{array} \right\} \text{ equal numbers.} \\ \text{II } \left\{ \begin{array}{l} 9 + x \\ 9 + x + s \end{array} \right\} \text{ equal numbers.} \end{cases}$$

A study of seventy or more individuals of this kind gives the impression that the small heterochromosome most often divides very late in the first division, but it is certain that there is considerable individual difference. In some cases nearly every anaphase of the first division shows  $s$  dividing; in others, it is rarely or never seen dividing in the first spindle, and as stated above, all of the various possibilities have been found in one individual.

### *Type IIb*

Fifteen out of the same 100 males of *Diabrotica soror* had two small heterochromosomes in addition to the eighteen ordinary chromosomes and the large heterochromosome  $x$ . These are shown in a spermatogonial plate (Fig. 49). The three heterochromosomes may also be seen in a growth stage (Fig. 50), a prophase of the first division (Fig. 51), lateral and polar views of the metaphase (Figs. 52 and 53) and an anaphase (Fig. 54). Fig. 55 is an equatorial plate of the second division. When two small heterochromosomes are present both may go to either

pole of the first division spindle, one to each pole, or one or both may divide as in Fig. 54. The resulting combinations in the spermatozoa are as follows:

$$\text{Equal numbers} \left\{ \begin{array}{l} \text{I} \left\{ \begin{array}{l} 9 \\ 9 + s \\ 9 + 2s \end{array} \right\} \text{ variable numbers.} \\ \text{II} \left\{ \begin{array}{l} 9 + x \\ 9 + x + s \\ 9 + x + 2s \end{array} \right\} \text{ variable numbers.} \end{array} \right.$$

*Type IIc*

Three out of the same 100 specimens had three small heterochromosomes, as shown in Fig. 56, a growth stage, 57 and 58, metaphases of the first division.

*Type IIId*

One individual had four such small heterochromosomes which may be seen in Figs. 59-65, growth stages and first spermatocytes. Here one may find all of the possibilities with respect to division and distribution of the small chromosomes. The possible combinations in the spermatozoa are therefore as follows:

$$\text{Equal numbers} \left\{ \begin{array}{l} \text{I} \left\{ \begin{array}{l} 9 \\ 9 + 1s \\ 9 + 2s \\ 9 + 3s \\ 9 + 4s \end{array} \right\} \text{ variable numbers.} \\ \text{II} \left\{ \begin{array}{l} 9 + x \\ 9 + x + 1s \\ 9 + x + 2s \\ 9 + x + 3s \\ 9 + x + 4s \end{array} \right\} \text{ variable numbers.} \end{array} \right.$$

There were no spermatogonial plates of type IIc and II<sub>d</sub> which could be counted, and in no case, though many ovaries have been fixed and sectioned and others examined with aceto-carmin, has it been possible to determine the number and character of the chromosomes in the female.

#### DIABROTICA 12-PUNCTATA

Exactly the same conditions as to the small heterochromosomes prevail in *Diabrotica 12-punctata* collected at Bryn Mawr, Pa., as in *Diabrotica soror* at Mountain View, Cal. Out of the first 100 males examined in October, 1907, 51 had no small heterochromosome, 35 had one, 11 had two, 2 had three and 1 had four, while in *Diabrotica soror* the numbers for the five corresponding classes were 48, 33, 15, 3, 1.

A few figures only will be given for *Diabrotica 12-punctata*. As in many other Coleoptera, spermatogonial equatorial plates in which the chromosomes are well enough separated for accurate counting are rarely found. The one shown for *Diabrotica soror*, type II<sub>b</sub>, in Fig. 49, was drawn from an aceto-carmin preparation in which the chromosomes had been separated by pressure on the cover-glass. Figs. 66 and 67 are spermatogonial plates of *Diabrotica 12-punctata*, type I and type II<sub>a</sub>, drawn from sections. There is some overlapping here, but no doubt as to the number in either plate. Growth stages for the five classes are shown in Figs. 68, 69, 70, 71 and 72. The larger size of both nucleus and chromosomes in Fig. 72 is due to its having been drawn with the same power from an aceto-carmin preparation. These figures also serve to show something of the diversity of form of the odd chromosome (*x*). When no small heterochromosome is present it usually is nearly spherical (Figs. 18 and 68). Where one or more of the small chromosomes are found, it is as a rule somewhat elongated (Figs. 69 and 70), often irregular in form (Fig. 72), or much elongated and bent in U-form. Whether this difference indicates some influence exerted by the presence of the smaller heterochromosomes, or marks the individuals containing the small chromosome as a separate species is not at present clear.

In both *Diabrotica soror* and *Diabrotica 12-punctata* the small heterochromosomes are usually quite closely associated with the larger one ( $x$ ) in the growth stages, but this is by no means invariably true. It is not at all unusual to find them separated in some cells and in one individual it was noted that the two were more often widely separated. (Figures to illustrate this have been thrown out for lack of space.)

Fig. 73 shows a metaphase of the first spermatocyte from the one individual of this species which had four small chromosomes. Figs. 74 and 75 are anaphases from the same section, from an individual with two small chromosomes, showing in one case (Fig. 74,  $s_1$  and  $s_2$ ) both dividing, in the other (Fig. 75) one dividing ( $s_2$ ) and the other ( $s_1$ ) passing undivided to the same pole with the odd chromosome ( $x$ ). In general, these small chromosomes are remarkably uniform in size. One case, however, was found among the aceto-carmin preparations where an unusually small one was constant for the individual (Figs 76-78). This very small chromosome was not found dividing in the first spermatocyte and it could not be followed in the second division. In one cyst the spireme was segmenting, later than usual, into dumb-bell shaped bivalents (Fig. 77), as in *Tenebrio molitor* (Stevens '05, Pl. 6, Figs. 177-179).

- As in the other species of *Diabrotica* it has not been possible to find favorable stages for counting the chromosomes in the female. One may be able to do this by breeding the insects and working with the tissues of the larva or pupa. Judging from similar cases where the female number is known (for the Coleoptera, *Elatér* I, Fig. 229, Pl. 13, Stevens '06, and *Photinus pennsylvanicus*, figures not yet published; *Anasa tristis* and other Hemiptera, Wilson '05 and '06; Pœcilopectera, Fig. 283, Pl. 8 and Fig. 294, Pl. 9, Boring '07), we must suppose that the female number for *Diabrotica vittata* is twenty-two and for *Diabrotica soror* and *Diabrotica 12-punctata*, type I, twenty. Since the small heterochromosomes seem to be as likely to go to the spermatozoa which receive the odd chromosome ( $x$ ) as to those which lack it, it would appear probable that the conditions with reference to the small heterochromosomes in the female are the same as in the male, and more-

over it is perfectly possible for more than four to occur in either male or female, as will be seen from the tables on p. 8.

#### DISCUSSION.

##### *Sex Determination*

For the present it is necessary to assume that the number of chromosomes in the female bears the same relation to the number in the male as in other cases among the Coleoptera and Hemiptera where an odd or unpaired heterochromosome is present in the male. The division products of the unpaired chromosome pass to one-half of the spermatozoa and these spermatozoa fertilize the eggs which develop into females; while the spermatozoa which lack the odd chromosome fertilize the eggs which produce males. This still seems to be as far as we can safely go in discussing the relation of the odd chromosome to sex determination. This chromosome is uniform in its behavior in the three species of *Diabrotica*, and it seems clear that it alone of the heterochromosomes described can have any connection with the determination of sex.

##### *The "Supernumerary" Chromosomes*

The small heterochromosomes in *Diabrotica 12-punctata* were first seen in some first spermatocyte spindles by Miss Anne M. Lutz of the Carnegie Institution of Experimental Evolution, Cold Spring Harbor, more than two years ago, but the matter was not followed up.

Prof. E. B. Wilson, in a recent communication (*Science*, n. s., vol. 26, no. 677, p. 870), has given the name "supernumerary" chromosomes to certain additional heterochromosomes in *Metapodius* (Hemiptera), and perhaps that name is as good as any other for the additional small heterochromosomes which appear in variable numbers in about 50 per cent of random collections of *Diabrotica soror* and *Diabrotica 12-punctata*. As in *Metapodius* the number of supernumeraries is constant for the individual. In *Metapodius* the supernumeraries are described as accompanying a pair of idiochromosomes with which they frequently unite to form a compound element in the second spermatocyte:

In the *Diabroticas* they are present with a larger unpaired heterochromosome, and there is no evidence that they are ever united with it. The most puzzling characteristic of the supernumeraries in the *Diabroticas* is the fact that they may in the same individual divide in either maturation division, and when two, three or four are present, each one may divide in either spermatocyte division, thus giving great diversity in the chromatin content of the spermatozoa. In *Metapodius* the supernumeraries are described as dividing in the first division.

Occasionally, as in Figs. 64 and 73 two of the supernumeraries seem to be paired in the metaphase of the first division, but this is probably accidental, as it is not constant in any individual.

The only other known case among the Coleoptera at all resembling this is that of the steel-blue flea-beetle, *Haltica chalybea*, which has a large and a small heterochromosome which are often widely separated in the metaphase of the first spermatocyte (figures not yet published). In the anaphase, however, the two heterochromosomes are found between the two daughter plates, and one goes to each second spermatocyte. This is merely a case of late pairing and the distribution of the division products of the two heterochromosomes to the spermatozoa is the same as in other cases of an unequal pair of heterochromosomes.

The first lot of *Diabrotica 12-punctata* were dissected out and all fixed together, so there was no opportunity to connect differences in the germ cells with differences in external characters of the insects, if such existed. In the California material obtained in December, 1906, and March, 1907, from Miss McCracken, each beetle, after dissecting out the testis or ovary, was preserved in alcohol, and later placed in the vial with its germ gland. In the December lot there was a difference of 1 mm. in the length of the Elytra, some measuring 4.5 mm., others 5.5 mm. All of the smaller beetles had the odd heterochromosome only, the others one supernumerary additional; and it was quite naturally supposed that there might be two distinct species or varieties, one of which had only the large unpaired heterochromosome, the other an unequal pair of heterochromosomes. In the March lot, one exception occurred—a small beetle had the additional small chro-

mosome, and there were several individuals of intermediate size which had one or more supernumeraries. In June, July and August, 100 males of *Diabrotica soror* were studied in California. The length of the elytron from origin to tip, measured to the nearest fourth of a millimeter in a straight line—not over the curve—was recorded for each beetle, the insects numbered and kept for future reference. After the hundred had been collected, measured and studied, they were arranged in series according to nuclear type. It was at once evident that the insects of type I and type II were about equally variable in size, and that all of the variations in fusion of spots occurred in each type. In fact no constant difference in external characters could be detected which might indicate two species. Thinking that possibly the variability in size might be different in the early and late broods, two lots of 100 each were collected November 1 and December 1, 1907, and measured without regard to the character of the germ cells. The results are given in a table below. Meanwhile 100 males of *Diabrotica 12-punctata* had been collected, measured, and the testes studied in aceto-carmin in October, 1907. This species is somewhat less variable than *Diabrotica soror*, but the two types with reference to the supernumerary chromosomes show the same kind of variations.

The variability in length of elytra of the different species and types is shown in Tables I and II.

TABLE I  
*Diabrotica soror.*

Length of elytron in mm.....	3	3.25	3.5	3.75	4	4.25	4.5	4.75	5	5.25	5.5	5.75	
Type I, 0 s.....	1		4		14	4	16	2	6		1		48
IIa, 1 s.....			1		8	5	10	1	6	1	1		33
IIb, 2 s.....					5	2	6		2				15
IIc, 3 s.....							1		2				3
IId, 4 s.....							1						1
June 23 to August 7.....	1	0	5	0	27	11	34	3	16	1	2		100
November 1.....			1	1	7	15	22	20	27	6	1		100
December 1.....				1	9	9	25	17	23	11	4	1	100
Total.....	1	0	6	2	43	35	81	40	66	18	7	1	300

TABLE II  
*Diabrotica 12-punctata*

Length of elytron in mm .....	3	3.25	3.5	3.75	4	4.25	4.5	4.75	5	5.25	5.5	5.75	
Type I, 0 s.....						2	7	7	23	9	1	2	51
IIa, 1 s.....				1			7	4	13	2	7	1	35
IIb, 2 s.....					1			1	9				11
IIc, 3 s.....							1		1				2
IId, 4 s.....								1					1
Total.....				1	1	2	15	13	46	11	8	3	100

It will be seen from the tables that *Diabrotica soror* is somewhat more variable and averages smaller in early summer than in late autumn; also that there is a possibility of two or three intergrading groups. The latter fact would not, however, seem to have any significance with reference to the supernumerary chromosomes, since in *Diabrotica 12-punctata* (Table II) the curve of variability is very steep with one mode at 5 mm. The 100 specimens of *Diabrotica 12-punctata* included in Table II were collected on some late goldenrod in one corner of a field on October 3, 4 and 9; the first 100 in Table I, in one rose garden in small collections extending over about six weeks. In both lots, most of the insects had recently emerged, and the conditions of temperature and nutrition under which they had developed could not have varied very greatly.

The one significant result so far as the supernumerary chromosomes are concerned is the parallel series of numbers for the five types of the two species—*Diabrotica soror*, 48, 33, 15, 3, 1 and *Diabrotica 12-punctata*, 51, 35, 11, 2, 1. Were it not for this parallelism of results in the two similar but geographically widely separated species,<sup>1</sup> one might suppose the presence of the supernumeraries to be accidental, due perhaps to an irregularity in the breaking up of the spireme or to imperfect metakinesis somewhere in the history of the male or female germ cells. The behavior of the supernumeraries in the growth stages of the spermatog-

<sup>1</sup> *Diabrotica 12-punctata* occasionally ranges into California, but belongs more especially to the eastern half of the United States, being perhaps most abundant in the Mississippi Valley.

cytes would suggest that they might have originated in a detached portion of the odd chromosome ( $x$ ), but such a supposition is not borne out by their later behavior in the maturation divisions, nor is there any evidence of an unequal pair among the other chromosomes indicating accidental separation of a part of one chromosome.

The only evidence I have that the supernumeraries might be chromosomes in the process of development or degeneration is the one individual (*Diabrotica* 12-punctata, No. 83 of the lot of 100 collected in October, 1907) in which one very small supernumerary was observed (Figs. 76-78). In other cases there seemed to be remarkable uniformity in size without regard to the number present.

If at some period in the past history of the race before the eastern and western species separated one supernumerary arose in any way, its peculiar habit of division, sometimes in one, sometimes in the other maturation division, may have given rise to the proportional numbers of the different types in the two species. Or it may still be possible, as was surmised earlier in the study, that (1) there will prove to be two distinct types (varieties or species) in each of the present species, one having the large unpaired heterochromosome only, the other having an unequal pair of heterochromosomes like that in *Haltica*, and that (2) the irregularities in time of division and the consequent peculiarities in number and distribution of the supernumeraries in *Diabrotica* are to be attributed to hybridism. If this should prove to be true it would indicate little or no hereditary value for these supernumeraries or for the smaller members of the unequal pair in other Coleoptera. A careful biometrical study of several external characters may bring to light some differences which can be associated with the presence or absence of the supernumeraries. The only other difference in the chromosomes of the two types seems to be a variation in the form of the odd chromosome ( $x$ ). In type I it is usually nearly spherical in growth stages, while in type II it is more or less elongated.

Until the material is investigated further, it hardly seems worth while to discuss at any greater length the hereditary significance of

the supernumerary chromosomes or the possible results of their irregular distribution. It however seemed advisable to publish the results which have been obtained, as considerable time must elapse before more material can be worked over; and it is to be hoped that another summer's work in California with breeding experiments and collections from different localities may furnish the data which are now lacking, and clear up the whole matter.

#### SUMMARY

1 *Diabrotica vittata* has twenty-one chromosomes, ten pairs and an unpaired heterochromosome which behaves like the odd chromosome in other Coleoptera and in the Orthoptera and Hemiptera homoptera, dividing in the second spermatocyte division, but not in the first. Synapsis occurs at the close of the synizesis stage. A chromatin nucleolus is present in all of the spermatids.

2 *Diabrotica soror* and *Diabrotica 12-punctata* both have in all cases nineteen chromosomes, nine pairs and an unpaired heterochromosome, which divides like that in *Diabrotica vittata*. About 50 per cent of the individuals examined have only nineteen chromosomes, the remaining 50 per cent have from one to four additional or "supernumerary" chromosomes which divide in either spermatocyte division, not in both, and may therefore give rise to from four to ten different kinds of spermatozoa with reference to their chromatin content, in the same individual. The percentage of individuals containing no supernumerary chromosome, one, two, three, or four supernumeraries, is nearly the same for the two species—48, 33, 15, 3, 1 for *Diabrotica soror* at Mountain View, California, and 51, 35, 11, 2, 1 for *Diabrotica 12-punctata* at Bryn Mawr, Pa. It has not as yet been possible to associate the different nuclear types with variations in any external character.

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Bryn Mawr, Pa.

NOTE—A part of the facts concerning the chromosomes in *Diabrotica soror* were given at the International Congress of Zoölogists in Boston, August 21, 1907.

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## DESCRIPTION OF PLATES

Figs. 1 to 48, 50, 54 and 66 to 71 were drawn from sections with 2 mm. obj. and 12 oc.; Figs. 49, 51 to 53 and 55 to 65 from aceto-carmin preparations with 2 mm. obj. and 6 oc.; Figs. 72 to 77 from aceto-carmin preparations with 2 mm. obj. and 12 oc. The magnification of all of the figures was then doubled with a drawing camera, and the plates reduced one-half.

### *Lettering used on Plates*

$\alpha$  = the unpaired, "odd" or "accessory" chromosome.

$n$  = the chromatin nucleolus of the spermatids.

$s$  = a "supernumerary" chromosome.

$s_1, s_2, s_3, s_4 = 1, 2, 3$  or 4 supernumerary chromosomes in the same individual.

## PLATE I

### *Diabrotica vittata*

Fig. 1 Spermatogonial metaphase, twenty-one chromosomes.

Fig. 2 First spermatocyte, synizesis stage.

Fig. 3 First spermatocyte, synapsis stage.

Fig. 4 First spermatocyte, postsynapsis stage.

Fig. 5 First spermatocyte, spireme stage.

Figs. 6 and 7 First spermatocytes, prophase.

Figs. 8 and 9 First spermatocytes, metaphase.

Figs. 10 and 11 Second spermatocytes, metaphase.

Figs. 12 to 15 Spermatids.

Fig. 16 Ripe spermatozoön.

### *Diabrotica soror. Type I*

Fig. 17 Spermatogonial metaphase, nineteen chromosomes.

Fig. 18 First spermatocyte, spireme stage.

Figs. 19 and 20 First spermatocytes, metaphase.

Fig. 21 First spermatocyte, anaphase.

Figs. 22 and 23 First spermatocyte, daughter plates.

Figs. 24 and 25 Second spermatocyte, rest stage.

Figs. 26 and 27 Second spermatocytes, metaphase.



7 m. S. del

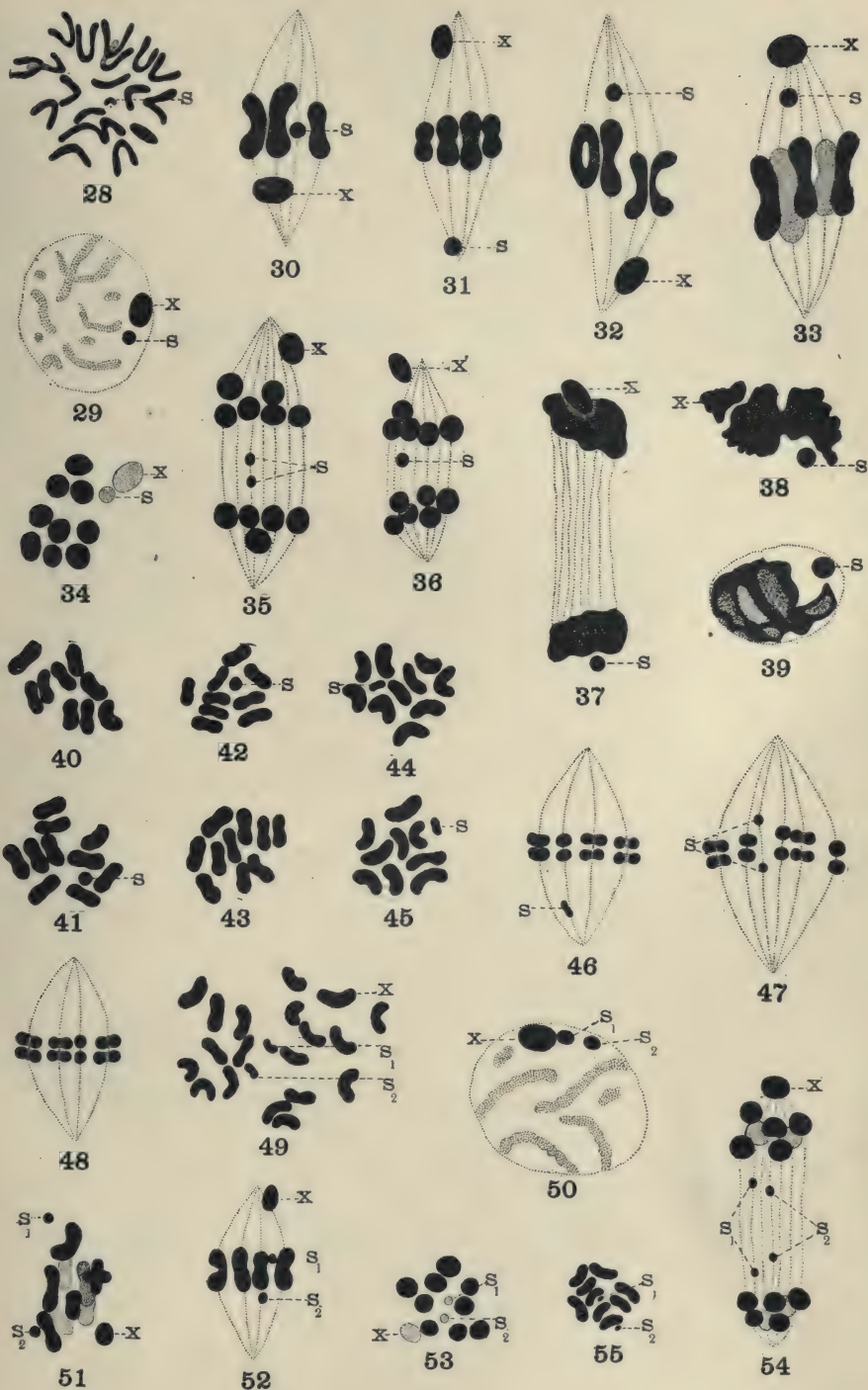
PLATE II

*Diabrotica soror. Type IIa*

- Fig. 28 Spermatogonial metaphase, twenty chromosomes.  
Fig. 29 First spermatocyte, spireme stage.  
Figs. 30 to 34 First spermatocytes, metaphase.  
Figs. 35 to 37 First spermatocytes, anaphase.  
Fig. 38 First spermatocyte, telophase.  
Fig. 39 Second spermatocyte, rest stage.  
Figs. 40 to 45 Second spermatocytes, metaphase, polar view.  
Figs. 46 to 48 Second spermatocytes, metaphase, lateral view.

*Type IIb*

- Fig. 49 Spermatogonial metaphase, twenty-one chromosomes.  
Fig. 50 First spermatocyte, spireme stage.  
Fig. 51 First spermatocyte, prophase.  
Figs. 52 and 53 First spermatocytes, metaphase.  
Fig. 54 First spermatocyte, anaphase.  
Fig. 55 Second spermatocyte, metaphase.



77 M. S. del

PLATE III

*Diabrotica soror. Type IIc*

Fig. 56 First spermatocyte, spireme stage.

Figs. 57 and 58 First spermatocytes, metaphase.

*Type IIId*

Figs. 59 and 60 First spermatocytes, spireme stage.

Fig. 61 First spermatocyte, prophase.

Figs. 62 to 64 First spermatocytes, metaphase.

Fig. 65 First spermatocyte, anaphase.

*Diabrotica 12-punctata*

Fig. 66 Spermatogonial metaphase, nineteen chromosomes.

Fig. 67 Spermatogonial metaphase, twenty chromosomes.

Fig. 68 First spermatocyte, spireme stage, no supernumerary.

Fig. 69 First spermatocyte, spireme stage, one supernumerary.

Fig. 70 First spermatocyte, spireme stage, two supernumeraries.

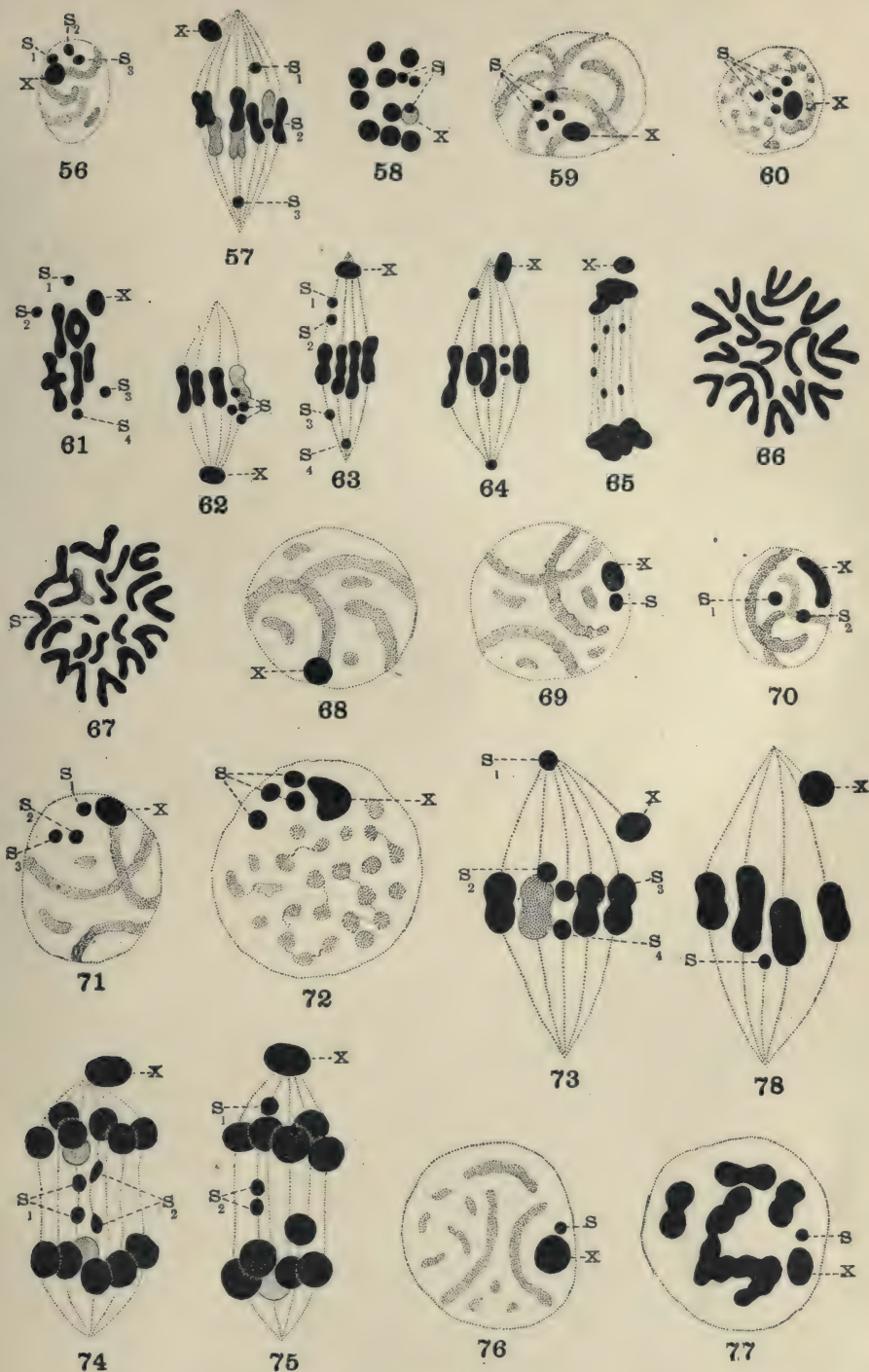
Fig. 71 First spermatocyte, spireme stage, three supernumeraries.

Fig. 72 First spermatocyte, spireme stage, four supernumeraries.

Fig. 73 First spermatocyte, metaphase, four supernumeraries.

Figs. 74 and 75 First spermatocytes, anaphase, two supernumeraries.

Figs. 76 to 78 First spermatocytes, unusually small supernumerary.



*n m s. del.*



## ON THE SPERMATOGENESIS OF THE EARWIG ANISOLABIS MARITIMA.<sup>1</sup>

HARRIET RANDOLPH.

The material for the examination of the germ cells of *Anisolabis maritima* came from a colony of these earwigs at Bryn Mawr. On account of the interest in the behavior of the chromosomes in the germ cells of insects, it seems desirable to add this group to the list of those that have been investigated recently in this country.

The material was preserved in Flemming's stronger fluid or in Gilson's mercurio-nitric solution and stained by Heidenhain's iron-hæmatoxylin method or with thionin.

A preliminary paper on the spermatogenesis of *Forficula auricularia* by Zweiger, '06, which appeared in 1906, contains references to the bibliography of the subject.

In the youngest stages found at Bryn Mawr each cyst contained several spermatogonia. In these cells (Fig. 1) in the resting stage there is a large spherical body which stains like chromatin.

In the equatorial plate of the dividing spermatogonia seen in polar view there are twenty-four chromosomes and a plasmosome which stains faintly (Fig. 2).

In the telophase of the last spermatogonial division two chromosome rods become connected with the plasmosome and remain condensed throughout the growth stages of the first spermatocytes.

Synizesis and synapsis stages are shown in Figs. 3 and 4, and the spireme in Fig. 5. At some time during the growth stages of the spermatocyte the heterochromosome pair separate from the plasmosome, forming a single rounded mass which lies free in the nuclear space (Figs. 6 and 7).

The splitting of the chromosomes is shown in Fig. 8. In Fig. 9 chromosomes from the prophase of the first spermatocyte are

<sup>1</sup> For the identification of the species I am indebted to the kindness of Mr. J. A. G. Rehn, of the Academy of Natural Sciences, Philadelphia, Penna.

shown, together with the heterochromosome pair and the plasmosome. All are from the same cyst. Figs. 10, 11 and 12 are from late prophases of the first spermatocyte division. The chromosomes arrange themselves into two groups at opposite poles. This is shown in Fig. 10, where the black bodies represent one group and those in light outline are  $180^\circ$  away, while the one in heavy outline is approximately at the equator. Figs. 11 and 12 show a centrosome close to each group which had apparently moved with its centrosome to that position. That the final position of any chromosome at one or the other of the poles is due to the centrosome in whose sphere of influence it happens to lie is suggested by the fact that occasionally seven chromosomes are at one pole and only five at the other.

In the equatorial plate of the first spermatocyte division there are normally twelve chromosomes (Fig. 13). In two or three earwigs a few cells of a cyst show eleven, thirteen, sixteen or nineteen chromosomes in the equatorial plate of this division; but there are also present in these cysts tripolar or multipolar spindles which probably explain the irregularity. There are also occasionally giant nuclei with double the normal number of chromosomes. In one case of an abnormal spindle it is known that the material came from an earwig that had very recently moulted, and it is possible that there is a connection between the two facts (Riddle, '08). In one instance a tripolar spindle was observed also in a spermatogonial division.

In the anaphase of the first spermatocyte division the heterochromosome pair are late in dividing and lag behind the others (Figs. 15, 16, 17). They are about equal in size. They finally separate (Figs. 18, 19, 20), one going to each pole of the spindle, and pass into the prophase of the second spermatocyte division (Fig. 21). Here again the chromosomes show a tendency to arrange themselves at the poles. Fig. 22 shows the metaphase and Fig. 23 the equatorial plate of the second spermatocyte division with twelve chromosomes. Figs. 24 and 25 are from stages in the anaphase. The earliest stages of the spermatids are shown in Figs. 26, 27, 28 and 29, where the behavior of the archoplasm and the change in position of the centrosome can be seen. Fig. 32 shows the condensed chromatin body in

the young spermatozoa. The older spermatozoa arrange themselves in bundles by inserting their heads into a cyst cell.

The material which is to form the spindle fibers is conspicuous at an early stage and is very considerable in amount. It forms another layer of fibers around the spindle proper (Figs. 5, 21, 22).

Something like the "mitosoma" described for *Forficula* by Zweiger, '06, is present in *Anisolabis* (Fig. 1), although the form is apparently unlike in the two species. It is traceable possibly from the spermatogonium to the spermatid; but as it does not stain with thionin after an early stage, and as it is very small and there are many granules in the iron-hæmatoxylin preparation, it is not by any means certain that the structures observed are one and the same throughout the series (Fig. 20).

The somatic chromosome number, found in the cells of the egg follicle, is twenty-four (Fig. 30). The material was not favorable for the examination of the ova. Very few were found in division stages and only one was cut so that its chromosomes could be counted. In this only equatorial plate observed the number of the chromosomes is twenty-four.

The characteristic structure of the male germ cells of *Anisolabis maritima* is an equal heterochromosome pair which are present possibly in the spermatogonia although they are not distinguishable from the other chromosomes in the spermatogonial divisions. In any case, it is formed anew in the telophase of the last spermatogonial division. It remains condensed during the growth stages of the first spermatocyte and divides equally in the first spermatocyte division, lagging behind the other chromosomes in the anaphase. It is not evident in the second spermatocyte division but there is a condensed chromatin body in the spermatids. This equal heterochromosome pair appears to be like the equal pair of idiochromosomes found by Wilson, '05, in *Nezara* and the equal heterochromosome pair of Stevens, '06, in *Lepidoptera*.

BRYN MAWR COLLEGE,  
June 2, 1908.

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## EXPLANATION OF PLATE I.

All figures were drawn with Zeiss camera lucida, 2 mm. oil immersion objective, 12 ocular, enlarged 2 diameters with a drawing camera and reduced the same.

FIG. 1. Spermatogonium, resting stage. *m* (?), the "mitosoma" of Zweiger.

FIG. 2. Spermatogonium, equatorial plate. *p*, the plasmosome.

FIG. 3. First spermatocyte, synizesis stage. *hc*, the heterochromosome.

FIG. 4. First spermatocyte, synapsis stage.

FIG. 5. First spermatocyte, spireme stage.

FIG. 6. First spermatocyte, the heterochromosome pair partially separated from the plasmosome.

FIG. 7. First spermatocyte, the heterochromosome pair separated from the plasmosome.

FIG. 8. First spermatocyte, the chromosomes partially split.

FIG. 9. First spermatocyte, prophase; the forms of the chromosomes, all from the same cyst.

FIG. 10. First spermatocyte, prophase; the six chromosomes in solid black are at one pole and the five in outline at the opposite pole; the one in heavy outline is at the equator.

FIGS. 11 and 12. First spermatocyte, prophase; the chromosomes on the way to opposite poles under the influence of the centrosomes.

FIG. 13. First spermatocyte, equatorial plate.

FIG. 14. First spermatocyte, metaphase.

FIG. 15. First spermatocyte, early anaphase.

FIGS. 16 and 17. Later anaphase stages.

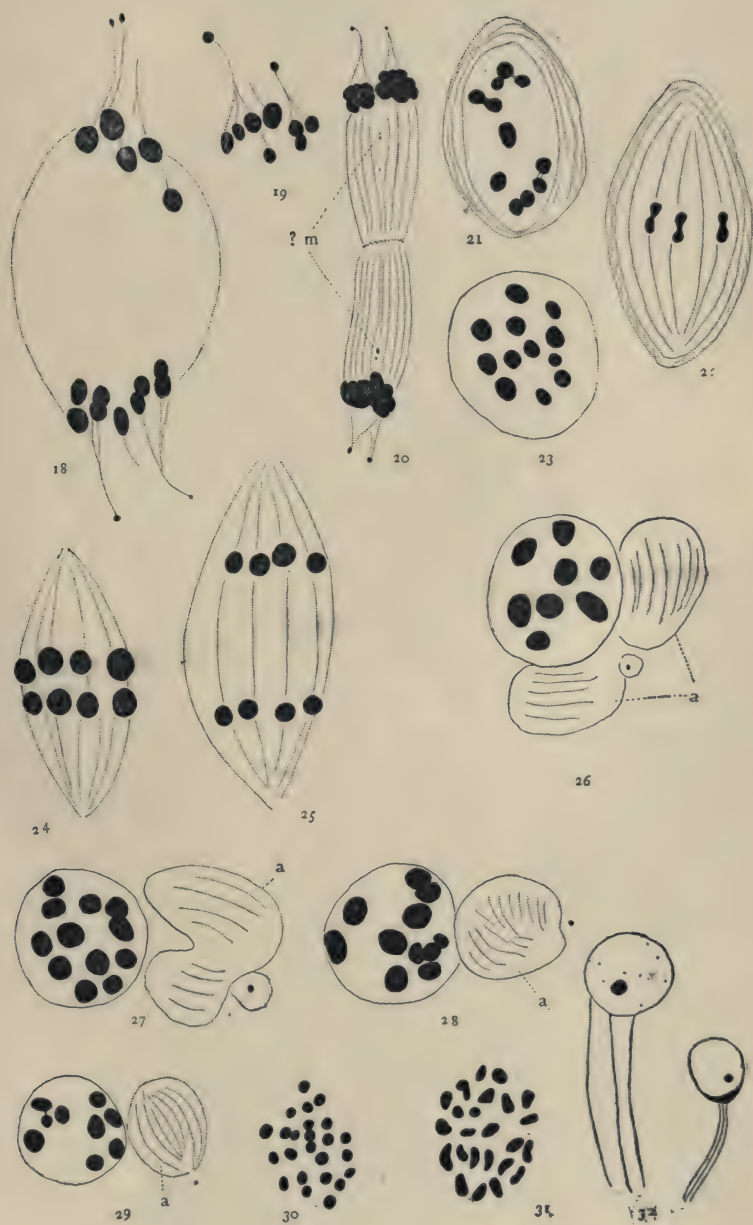






## EXPLANATION OF PLATE II.

- FIGS. 18 and 19. Late anaphase stages.
- FIG. 20. Telophase; *pm*, the "mitosoma" of Zweiger.
- FIG. 21. Second spermatocyte, prophase.
- FIG. 22. Second spermatocyte, metaphase.
- FIG. 23. Second spermatocyte, equatorial plate.
- FIGS. 24 and 25. Second spermatocyte, anaphase stages.
- FIGS. 26, 27, 28, 29. Spermatids, showing archoplasma (*a*) in different stages, and the movement of the centrosome.
- FIG. 30. Somatic cell of female, equatorial plate.
- FIG. 31. Ovum, equatorial plate.
- FIG. 32. Young spermatozoa containing condensed chromatin.





## THE CHROMOSOMES IN CROSS-FERTILIZED ECHINOID EGGS.

D. H. TENNENT.

In experiments which I carried on during the summer of 1907<sup>1</sup> crosses were made between several echinoids, namely, by the fertilization of:

1. The egg of the spatangoid *Moiria atropos* with the sperm of the sand-dollar *Mellita pentapora*.
2. The egg of *Moiria* with the sperm of the sea urchin *Toxopneustes variegatus*.
3. The egg of *Moiria* with the sperm of the sea urchin *Arbacia punctulata*.
4. The egg of *Toxopneustes* with the sperm of *Moiria*.
5. The egg of *Toxopneustes* with the sperm of *Mellita*.
6. The egg of *Arbacia* with the sperm of *Moiria*.
7. The egg of *Arbacia* with the sperm of *Mellita*.
8. The egg of *Mellita* with the sperm of *Moiria*.

The work was undertaken primarily with the object of obtaining material for a cytological study of cross-fertilized eggs and secondarily for the purpose of making a comparison, based especially upon the character of the skeleton, between larval forms.

In this paper I shall consider some of the earlier phenomena exhibited, in brief, the prophases and early metaphases of division, in two of the crosses, (1),  $\frac{\textit{Moiria} \text{ } \sigma}{\textit{Toxopneustes} \text{ } \text{f}}$  and (2),  $\frac{\textit{Moiria} \text{ } \sigma}{\textit{Arbacia} \text{ } \text{f}}$ , reserving the consideration of later stages, of the other crosses, and a general discussion of the results for a latter contribution.

The method of effecting the cross-fertilization was the exceedingly simple one of allowing the eggs, after their removal from the ovary, to stand for several hours in sea water, the water being changed occasionally, and at the most favorable time, which was

<sup>1</sup> I wish to express my thanks to the Hon. George M. Bowers, U. S. Commissioner of Fisheries, for the privilege of working in the Beaufort Laboratory and to Mr Henry D. Aller, director of the laboratory, for many courtesies extended to me. I am also indebted to Dr. Bartgis McGlone for information regarding the artificial fertilization of *Moiria* eggs.



clei, formation of the amphiaster, etc., is then approximately the same as in *Toxopneustes* eggs fertilized with *Toxopneustes* sperm.

The chromosomes as seen in a polar view of the equatorial plate of  $\frac{\textit{Toxopneustes} \text{ } \sigma}{\textit{Toxopneustes} \text{ } \varphi}$  eggs are shown in Figs. 1 and 2.

They are seen to have the appearance of rather long, slender, and somewhat bent rods. By comparing these two figures it may be seen that variations in the form of the chromosomes, which are correlated with slight differences in the ages of the plates, are evident.

A corresponding view of the chromosomes in a section of a  $\frac{\textit{Maira} \text{ } \sigma}{\textit{Maira} \text{ } \varphi}$  egg is shown in Fig. 3.

Some differences are apparent, but in general the size, form, etc., of the chromosomes in this plate are so like those of the *Toxopneustes* egg that one need scarcely venture to hope to be able to identify the chromosomes of maternal and paternal origin in the cross-fertilized eggs.

An examination of sections such as are illustrated in Figs. 4 and 5 convinces me that we have here a mixture of the two sorts, but I find myself unwilling or perhaps unable to distinguish the chromosomes of either origin.

Some interesting variations from the normal were found in one series of  $\frac{\textit{Maira} \text{ } \sigma}{\textit{Toxopneustes} \text{ } \varphi}$  eggs in which more than one spermatozoön had entered the egg. Two different results are shown in Figs. 9-12 and Text Fig. 1.

In one case the extra sperm-nucleus is seen moving toward the segmentation nucleus. Its aster has divided while the centrosome of the future cleavage amphiaster is still single (Fig. 9). Later the second sperm nucleus seems about to fuse with the segmentation nucleus while the centers of the regular cleavage amphiaster have separated (Fig. 10). In some cases fusion between the two nuclei takes place; in others (Fig. 11), the fibers from one of the sperm asters enter the nucleus and the chromosomes become differentiated in the network before the cleavage asters have well separated.

In the other case (Fig. 12 and Text Fig. 1), chromosomal

differentiation and separation of the cleavage centers had gone on to a considerable extent before the additional spermatozoön had entered the egg. Here the two amphiasters are seen side by side. In Text Fig. 1 what may possibly be sperm tails are seen lying within a fertilization cone, although the entrance of the tail

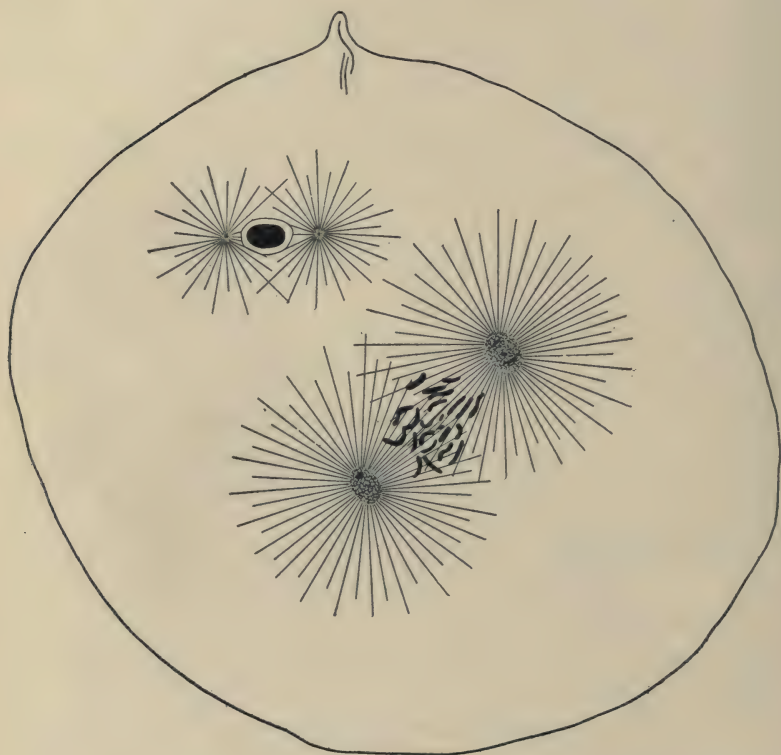


FIG. 1. *Toxopneustes* egg  $\times$  *Moira* sperm. (Drawn to same scale and reduced slightly more than are plate figures.) Segmentation nucleus dividing. Extra sperm nucleus in prophase.

in the fertilization of the echinoderm egg is contrary to the general belief.

These cases ought to prove of interest in further investigation along the lines laid down by Boveri in his recent contribution on dispermic sea urchin eggs (Zellen-Studien, Heft 6).

B. THE *Moir*a ♂ *Arbacia* ♀ CROSS.

In effecting this cross the *Arbacia* eggs were allowed to stand in sea water for seven hours, the water being changed every hour, and then fertilized. The controls gave no segmentation.

Cleavage began about forty minutes later ; again approximately as in normally fertilized eggs, in both cases being slightly hastened or retarded by variations in the temperature of the water.



FIG. 2. *Arbacia* egg  $\times$  *Moir*a sperm. Egg outline omitted, otherwise drawn to same scale and reduced as are plate figures. Chromosomes scattered throughout cytoplasm. *Arbacia* chromosomes and *Moir*a chromosomes may be distinguished from one another by size.

The sections of eggs of this cross are perhaps of greater interest than those of the *Moir*a-*Toxopneustes* cross because of the fact that the chromosomes of the two species are of sufficient difference in form to be distinguished from one another.

Fig. 6 shows the chromosomes of an equatorial plate of an *Arbacia* ♂  
*Arbacia* ♀ egg. The chromosomes here are seen to be short, slightly bent rods. These are quite different in form from those already mentioned in the equatorial plate of *Moir*a (Fig. 3), where the chromosomes are longer and comparatively more slender.

The sections of the *Moir*a-*Arbacia* cross-fertilized eggs giving a polar view of the equatorial plates (Figs. 7 and 8), show a mixture of short and long forms probably indicating *Arbacia* and *Moir*a chromosomes respectively. These differences in form are evident in the equatorial plates of both the first and second cleavages, which is as far as I have carried the observations.

The differences in form are less evident in the late metaphases or early anaphases when the daughter chromosomes are drawn out, behaving like substances with a high surface tension, and then contracting during the late anaphases, into much shorter rods.

In both of the crosses, but especially in sections of eggs of the *Moir*a-*Arbacia* cross, an interesting phenomenon may be noted (Text Fig. 2).

In eggs in which the daughter nuclei are in the resting condition succeeding the first division, the cytoplasm contains many deeply staining rods. The nucleus at this time does not take the chromatin stain and appears like an empty vesicular structure.

In eggs, of the same lot and on the same slides, in which the fibers of the second amphiaster have begun to form, the nucleus again takes the stain and shows the chromatic net, while the cytoplasm is seen to be free from the bodies described.

These structures have puzzled me not a little, but I have finally reached the conclusion that the eggs in which they occur are degenerating. Even though this be true it is difficult to explain the simulation or perhaps occurrence of longitudinal and transverse divisions of these chromosomes lying free in the cytoplasm.

#### SUMMARY.

This paper deals with observations made on sections of cross-fertilized eggs of two kinds: (1) *Toxopneustes* eggs fertilized with *Moir*a sperm, (2) *Arbacia* eggs fertilized with *Moir*a sperm.

The results of the study may be summarized as follows :

1. The equatorial plate of the *Moiria-Toxopneustes* cross shows a mixture of two kinds of chromosomes not sufficiently unlike one another to enable a positive distinction between the two.

2. The equatorial plate of the *Moiria-Arbacia* cross shows a mixture of two kinds of chromosomes, one variety long, the other variety short. These differences in form are correlated with the spermatozoön and the egg respectively.

BRYN MAWR COLLEGE,  
March, 1908.

## EXPLANATION OF PLATE I.

All of the figures were drawn with the aid of a camera and Zeiss compensation ocular 12 and 2 mm. Apochromatic oil immersion objective. They were enlarged two diameters with a drawing camera and have been reduced to one half in reproduction.

FIG. 1. *Toxopneustes* egg  $\times$  *Toxopneustes* sperm. Equatorial plate. Polar view.

FIG. 2. Same as Fig. 1.

FIG. 3. *Moiria* egg  $\times$  *Moiria* sperm. Eq. pl.

FIG. 4. *Toxopneustes* egg  $\times$  *Moiria* sperm. Eq. pl.

FIG. 5. Same as Fig. 4.

FIG. 6. *Arbacia* egg  $\times$  *Arbacia* sperm. Eq. pl.

FIG. 7. *Arbacia* egg  $\times$  *Moiria* sperm. Eq. pl.

FIG. 8. Same as Fig. 7.

FIG. 9. *Toxopneustes* egg  $\times$  *Moiria* sperm. Segmentation nucleus with centrosome undivided. Extra sperm nucleus with aster divided.

FIG. 10. *Toxopneustes* egg  $\times$  *Moiria* sperm. Segmentation nucleus with centrosome divided. Extra male nucleus, with its aster divided, in contact with segmentation nucleus.

FIG. 11. *Toxopneustes* egg  $\times$  *Moiria* sperm. Segmentation nucleus with centrosome divided. Fibers from aster of extra sperm nucleus extending into the segmentation nucleus.

FIG. 12. *Toxopneustes* egg  $\times$  *Moiria* sperm. Segmentation nucleus and extra sperm nucleus lying side by side and both preparing for division.

